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(54) Title: IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

(57) Abstract: The present invention concerns a method for altering characteristics of a plant. The invention describes the identification of genes that are upregulated or downregulated in transgenic plants overexpressing E2Fa/DPa and the use of such sequences to alter plant characteristics. A preferred way for altering characteristics of a plant comprises modifying expression of one or more nucleic acid sequences and or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one or more of SEQ ID NO 1 to 2755. Some of the genes identified in the present invention have an E2Fa target consensus sequence in their 5' upstream region. The identified genes play a role in a variety of biological processes, such as DNA replication, cell wall biosynthesis, nitrogen and/or carbon metabolism, transcription factors etc.

# IDENTIFICATION OF NOVEL E2F TARGET GENES AND USE THEREOF

The present invention concerns altering plant characteristics. More particularly, the present invention relates to identification of genes and proteins involved in E2Fa/DPa-mediated processes and further relates to use of such genes and proteins for altering characteristics in plants.

The present invention concerns a method for altering one or more plant characteristics, whereby the altered plant characteristic is selected from altered development, altered plant growth, altered, for example increased, plant yield and/or biomass, biochemistry, physiology, architecture, metabolism, survival capacity or stress tolerance by modifying expression of one or more of the genes according to the present invention and/or by modifying levels and/or activity of the proteins encoded by these genes. The present invention also concerns genetic constructs for performing the methods of the invention and to plants or plant parts obtainable by the methods of the present invention, which plants have altered characteristics compared to their otherwise isogenic counterparts. The invention also extends to recombinant nucleic acids and the use thereof in the methods according to the invention.

Growth, development and differentiation of higher organisms are controlled by a highly ordered set of events called the cell cycle (Morgan, 1997). Cell division and cell growth are operated by the cell cycle, which ensures correct timing and high fidelity of the different transition events involved. Cell cycle regulation at both G1→S and G2→M phase transitions depends on the formation of appropriate protein complexes and both transitions are believed to be the major control points in the cell cycle. The cell's decision to proliferate and synthesize DNA and ultimately to divide is made at the G1→S restriction point in late G1. Overcoming this point of no return requires the cell's competence to initiate DNA synthesis as well as the expression of S-phase genes. Transcription of S-phase-specific genes requires binding to the DNA of an E2F transcription factor. Dimerisation of E2F with DP is a prerequisite for high affinity binding to the E2F consensus DNA binding site (A/T)TT(G/C)(G/C)C(G/C)(G/C) (SEQ ID NO 2775), for example (TTT(C/G)(C/G)CGC), that can be found in the promoters of genes involved in DNA replication, repair, checkpoint control and differentiation (Ren et al., 2002; Weinmann et al., 2001; Kel et al., 2001). Variants of this consensus sequence as well as other locations of this consensus sequences are also found. The heterodimeric E2F/dimerization partner (DP) transcription factor also regulates the promoter activity of multiple genes, which are essential for DNA replication and cell cycle control (Helin, 1998; Müller and Helin, 2000). E2F transcription factors are critical effectors of the decision to pass the restriction point and to allow the cell to proceed in S-phase.

In the *Arabidopsis* genome, 3 E2F (E2Fa, E2Fb, and E2Fc) and 2 DP genes (DPa and DPb) are present (Vandepoele et al., 2002). The phenotypic analysis of plants overexpressing E2Fa and DPa was described recently (De Veylder et al., 2002). Microscopic analysis revealed that E2Fa/DPa overproducing cells underwent ectopic cell division or endoreduplication, depending on the cell type. Whereas extra cell divisions resulted in cells being smaller than those seen in the same tissues of control plants, extra endoreduplication caused formation of giant nuclei. RT-PCR demonstrated that expression levels of genes involved in DNA replication (CDC6, ORC1, MCM, DNA pol α) were strongly upregulated in plants overexpressing E2Fa and DPa (De Veylder et al., 2002).

The present invention provides genes having altered expression levels in plants overexpressing E2Fa and DPa relative to expression levels in corresponding wild type plants. Furthermore, the present invention provides means to modulate expression of these genes, which in turn allows for modulation of the biological processes that they control. The present invention provides methods to mimic E2F/DP level and/or activity by manipulating downstream factors involved in E2F/DP pathways. This strategy allows a fine-tuning of the effects of E2Fa/DPa. Whereas overexpression of E2Fa or DPa or both can be pleiotropic and/or can have pleiotropic effects, it is the invention provides methods to alter plant characteristics in a more controlled and targeted way, by using the E2F/DP target genes as defined by the present invention. Modulation of particular biological processes is now possible and may give rise to plants having altered characteristics, which may have particularly useful applications in agriculture and horticulture.

Therefore, according to the present invention, there is provided a method to alter one or more plant characteristics, comprising modifying, in a plant, expression of one or more nucleic acids and/or modifying level and/or activity of one or more proteins, which nucleic acids or proteins are essentially similar to any one of SEQ ID NO 1 to 2755, and wherein said one or more plant characteristics are altered relative to corresponding wild type plants.

The inventors designed a microarray experiment, comparing transcript levels of more than 4579 genes of wild type and transgenic *Arabidopsis* lines overexpressing E2Fa/DPa. Surprisingly, the inventors found that particular genes are up or down regulated in E2Fa-DPa overexpressing plants. The sequences which were at least 1.3 times upregulated or downregulated, are represented with their MIPS (Munich information center for protein sequences) accession number MATDB database http://mips.gsf.de/proj/thal/db/index.html) in Tables 4 and 5. Sequences which were at least 2-fold upregulated or 2-fold downregulated are shown in Tables 1 and 2, respectively. Further classification of these genes according to their function is provided in Tables 1 and 2. Promoter analysis of these genes allowed for the

identification of genes under the direct control of E2Fa and/or DPa proteins and genes that are indirectly controlled by the E2Fa/DPa complex. Examples of mechanisms for such indirect control include, (i) recognition by E2F/DP of other sequence elements that diverge from the consensus recognition site; (ii) possible association of E2F/DP with other DNA binding proteins capable of recognizing other DNA elements; and (iii) sequential transcription activation of a first gene capable of regulating transcription of a second gene. It is to be understood that having an E2F target sequence is not a prerequisite to be regulated by E2F.

The gene that corresponds to the sequence deposited under the MIPS database accession number At1g57680 is an example of a gene, which is likely to be indirectly controlled by the E2Fa/DPa complex. This gene is of unknown function. It was surprising to find this unknown gene and the other genes of Tables 1, 2, 4 and 5 to be involved in E2Fa/DPa controlled processes. The genes according to the present invention are represented herein with their nucleic acid sequence and corresponding amino acid sequence as set forth in SEQ ID NO 1 to 2755.

Preferably expression and/or level and/or activity of one of the genes and/or proteins according to any of SEQ ID NO 1 to 2755 is modified. Alternatively expression and/or level and/or activity of one or more of those genes and/or proteins is modified. According to a further embodiment one or more gene/and or proteins of the same functional category as presented in Table 1 or Table 2, are modified.

The term "modifying expression" relates to altering level (increasing expression or decreasing expression) or altering the time or altering the place of expression of a nucleic acid. The term "modified" as used herein is used interchangeably with "altered" or "changed".
Modified expression (or level or activity) of a sequence essentially similar to any one of SEQ ID NO 1 to 2755 encompasses changed expression (or level or activity) of a gene product, namely a polypeptide, in specific cells or tissues. The changed expression, activity and/or levels are changed compared to expression, activity and/or levels of the gene or protein essentially similar to any one of SEQ ID NO 1 to 2755 acid in corresponding wild-type plants. The changed gene expression may result from changed expression levels of an endogenous gene essentially similar to any one of SEQ ID NO 1 to 2755 acid and/or may result from changed expression levels of a gene essentially similar to SEQ ID NO 1 to 2755 acid previously introduced into a plant. Similarly, changed levels and/or activity of a protein essentially similar to any one of SEQ ID NO 1 to 2755 acid may be due to changed expression of an endogenous nucleic acid/gene and/or due to changed expression of nucleic acid/gene previously introduced into a plant.

Modified expression of a gene/nucleic acid and/or increasing or decreasing activity and/or levels of a gene product may be effected, for example, by chemical means and/or recombinant means.

Advantageously, modified expression of a nucleic acid according to the invention and/or modified activity and/or levels of a protein according to the invention may be effected by chemical means, i.e. by exogenous application of one or more compounds or elements capable of modifying activity and/or levels of the protein and/or capable of modifying expression of a nucleic acid/gene according to the invention. The term "exogenous application" as defined herein is taken to mean the contacting or administering of a suitable compound or element to plant cells, tissues, organs or to the whole organism. The compound or element may be exogenously applied to a plant in a form suitable for plant uptake (such as through application to the soil for uptake via the roots, or in the case of some plants by applying directly to the leaves, for example by spraying). The exogenous application may take place on wild-type plants or on transgenic plants that have previously been transformed with a nucleic acid/gene according to the present invention or with another transgene.

Suitable compounds or elements include proteins or nucleic acids according to SEQ ID NO 1 to 2755 or proteins or nucleic acids essentially similar to SEQ ID NO 1 to 2755. Essentially similar proteins or nucleic acids are, homologues, derivatives or active fragments of these proteins and/or portions or sequences capable of hybridizing with these nucleic acids. The exogenous application of yet other compounds or elements capable of modifying levels of factors that directly or indirectly activate or inactivate a protein according to the present invention will also be suitable in practicing the invention. These compounds or elements also include antibodies that can recognize or mimic the function of the proteins according to the present invention. Such antibodies may comprise "plantibodies", single chain antibodies, IgG antibodies and heavy chain camel antibodies, as well as fragments thereof. Additionally or alternatively, the resultant effect may also be achieved by the exogenous application of an interacting protein or activator or an inhibitor of the gene/gene product according to the present invention. Additionally or alternatively, the compound or element may be a mutagenic substance, such as a chemical selected from any one or more of: N-nitroso-N-ethylurea, ethylene imine, ethyl methanesulphonate and diethyl sulphate. Mutagenesis may also be achieved by exposure to ionising radiation, such as X-rays or gamma-rays or ultraviolet light. Methods for introducing mutations and for testing the effect of mutations (such as by monitoring gene expression and/or protein activity) are well known in the art.

Therefore, according to one aspect of the present invention, there is provided a method for altering plant characteristics, comprising exogenous application of one or more compounds or elements capable of modifying expression of a gene and/or capable of modifying activity and/or levels of a protein according to the present invention.

Additionally or alternatively, and according to a preferred embodiment of the present invention, modified of expression of a nucleic acid and/or modified of activity and/or levels of a protein, wherein these nucleic acids or proteins are essentially similar to any of SEQ ID NO 1 to 2755, may be effected by recombinant means. Such recombinant means may comprise a direct and/or indirect approach for modifying expression of a nucleic acid and/or for modifying activity and/or levels of a protein.

Therefore there is provided by the present invention, a method to alter plant characteristics, comprising modifying gene expression and/or protein levels and/or protein activity, which modification may be effected by recombinant means and/or by chemical means and wherein said gene and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755.

An indirect recombinant approach may comprise for example introducing, into a plant, a nucleic acid capable of increasing or decreasing activity and/or levels of the protein in question (a protein essentially similar to any one of SEQ ID NO 1 to 2755) and/or capable of increasing or decreasing expression of the gene in question (a gene essentially similar to any one of SEQ ID NO 1 to 2755). Examples of such nucleic acids to be introduced into a plant, are nucleic acids encoding transcription factors or activators or inhibitors that bind to the promoter of a gene or that interact with a protein essentially similar to any one of SEQ ID NO 1 to 2755. Methods to test these types of interactions and methods for isolating nucleic acids encoding such interactors include yeast one-hybrid or yeast two-hybrid screens.

Also encompassed by an Indirect approach for modifying activity and/or levels of a protein according to the present invention and/or expression of a gene according to the present invention, is the provision of a regulatory sequence, or the inhibition or stimulation of regulatory sequences that drive expression of the native gene in question or of the transgene in question. Such regulatory sequences may be introduced into a plant. For example, the nucleic acid introduced into the plant is a promoter, capable of driving the expression of the endogenous gene essentially similar to any one of SEQ ID NO 1 to 2755.

A further indirect approach for modifying activity and/or levels and/or expression of a gene or protein according to the present invention in a plant encompasses modifying levels in a plant of

a factor able to interact with the protein according to the present invention. Such factors may include ligands of the protein according to the present invention. Therefore, the present invention provides a method for altering characteristics of a plant, when compared to the corresponding wild-type plants, comprising modifying expression of a gene coding for a protein which is a natural ligand of a protein essentially similar to any one of SEQ ID NO 1 to 2755. Furthermore, the present invention also provides a method to alter one or more plant characteristics relative to corresponding wild-type plants, comprising modifying expression of a gene coding for a protein which is a natural target/substrate of a protein essentially similar to SEQ ID NO 1 to 2755.

A direct and more preferred approach to alter one or more plant characteristics, comprises introducing into a plant a nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755, wherein said nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 is any one of SEQ ID NO 1 to 2755 or a portion thereof or sequences capable of hybridizing therewith and which nucleic acid preferably encodes a protein essentially similar to any one of SEQ ID NO 1 to 2755, which protein essentially similar to any one of SEQ ID NO 1 to 2755 is any one of SEQ ID NO 1 to 2755 or a homologue, derivative or active fragment thereof. The nucleic acid may be introduced into a plant by, for example, transformation.

In the context of the present invention the term "modifying expression" and modifying level and/or activity encompasses "enhancing or decreasing". Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and are for example overexpression driven by a strong promoter, the use of transcription enhancers or translation enhancers. The term "overexpression" of a gene refers to expression patterns and/or expression levels of said gene normally not occurring under natural conditions. Ectopic expression can be achieved in a number of ways including operably linking of a coding sequence encoding said protein to an isolated homologous or heterologous promoter in order to create a chimeric gene.

Alternatively and/or additionally, increased expression of a gene or increased activities and/or levels of a protein in a plant cell, is achieved by mutagenesis. For example these mutations can be responsible for the changed control of the gene, resulting in more expression of the gene, relative to the wild-type gene. Mutations can also cause conformational changes in a protein, resulting in more activity and/or levels of the protein.

Examples of decreasing expression of a gene are also well documented in the art and include for example: downregulation of expression by anti-sense techniques, RNAi techniques, small

interference RNAs (siRNAs), microRNA (miRNA), etc. Therefore according to a particular aspect of the invention, there is provided a method to alter characteristics of plants, including technologies that are based on for example the synthesis of antisense transcripts, complementary to the mRNA of a gene essentially similar to any one of SEQ ID NO 1 to 2755.

Another method for downregulation of gene expression or gene silencing comprises use of ribozymes, for example as described in WO9400012 (Atkins *et al.*), WO9503404 (Lenee *et al.*), WO0000619 (Nikolau *et al.*), WO9713865 (Ulvskov *et al.*) and WO9738116 (Scott *et al.*).

Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by gene silencing strategies as described among others in the documents WO9836083 (Baulcombe and Angell), WO9853083 (Grierson *et al.*), WO9915682 (Baulcombe *et al.*) or WO9953050 (Waterhouse *et al.*).

Expression of an endogenous gene may also be reduced if the endogenous gene contains a mutation. Such a mutant gene may be isolated and introduced into the same or different plant species in order to obtain plants having altered characteristics. Also dominant negative mutants of a nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 can be introduced in the cell to decrease the level/and or activity of the endogenous corresponding protein.

Other methods to decrease the expression of a nucleic acid and/or activity and/or level of proteins essentially similar to any one of SEQ ID NO 1 to 2755 in a cell encompass, for example, the mechanisms of transcriptional gene silencing, such as the methylation of the promoter of a gene according to the present invention.

Modifying expression of the gene also encompasses altered transcript level of the gene. Altered transcript levels of a gene can be sufficient to induce certain phenotypic effects, for example via the mechanism of cosuppression. Here the overall effect of overexpression of a transgene is that there is less level and/or activity in the cell of the protein, which is encoded by the native gene showing homology to the introduced transgene.

Cosuppression is accomplished by the addition of coding sequences or parts thereof in a sense orientation into the cell. Therefore, according to one aspect of the present invention, the characteristics of a plant may be changed by introducing into a plant an additional copy (in full or in part) of a gene essentially similar to any one of SEQ ID NO 1 to 2755 already present in a host plant. The additional gene may silence the endogenous gene, giving rise to a phenomenon known as co-suppression.

According to the invention, "nucleic acid" or the "gene" essentially similar to any one of SEQ ID NO 1 to 2755 in a plant may be the wild type gene, i.e. native or endogenous or heterologous, i.e. derived from another individual plant or plant species. The gene (transgene) may be substantially modified from its native form in composition and/or genomic environment through deliberate human manipulation. This transgene can be introduced into a host cell by transformation techniques. Also expression of the native genes can be modified by introduction in the plant of regulatory sequences capable of altering expression of the native gene, as described above.

The term "modifying activity" relates to enhancing, decreasing or altering time or place of activity of a protein or polypeptide. According to the invention, the "protein" or the "polypeptide" may be the wild type protein, i.e. native or endogenous, or alternatively, the protein may be heterologous, i.e. derived from another individual or species.

The term "essentially similar to" in relation to a protein of the present invention as used herein includes variants such as homologues, derivatives and functional fragment thereof. The term "essentially similar to" in relation to a gene includes variants such as at least a part of the gene in question; a complement of the gene; RNA, DNA, a cDNA or a genomic DNA corresponding to the protein or gene; a variant of the gene due to the degeneracy of the genetic code; a family member of the gene or protein; an allelic variant of the gene or protein; and different splice variant of the gene or protein and variants that are interrupted by one or more intervening sequences. Advantageously, nucleic acids or proteins essentially similar to nucleic acids and the proteins according to any of SEQ ID NO 1 to 2755 may be used in the methods of the present invention. These variant nucleic acids and variant amino acids are described further below.

Any variant of a particular protein according to the present invention is a variant, which upon construction of a phylogenetic tree with that particular protein, tends to cluster around the particular protein which is any one of SEQ ID NO 1 to 2755. Such a phylogenetic tree can be constructed with alignments of amino acid sequences or with nucleic acid sequences. A person skilled in the art could readily determine whether any variant in question falls within the definition of a "a nucleic acid or protein essentially similar to any one of SEQ ID NO 1 to 2755". Hereto the man skilled in the art would use known techniques and software for the making of such phylogenetic trees, such as a GCG, EBI or CLUSTAL package, or Align X, using default parameters. Advantageously, the methods according to the present invention may also be practised using such variants.

Any variant suitable for use in the methods according to the invention may readily be determined using routine techniques, such as by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the fragment to be tested for functionality.

A first example of such variants are "homologues" of the proteins of the present invention, which homologues encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or additions relative to the protein in question and having similar biological and functional activity as an unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984).

The homologues useful in the method according to the invention may have at least 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48% or 50% sequence identity or similarity (functional identity) to the unmodified protein, alternatively at least 52%, 54%, 56%, 58% or 60% sequence identity or similarity to an unmodified protein, or alternatively at least 62%, 64%, 66%, 68% or 70% sequence identity or similarity to an unmodified protein. Typically, the homologues have at least 72%, 74%, 76%, 78% or 80% sequence identity or similarity to an unmodified protein, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89% sequence identity or similarity, further preferably at least 90%, 91%, 92%, 93% or 94% sequence identity or similarity to an unmodified protein, further preferably at least 95% 96%, 97%, 98% or 99% sequence identity or similarity to an unmodified protein. This % identity can be calculated using the Gap program in the WISCONSIN PACKAGE version 10.0-UNIX from Genetics Computer Group, Inc based on the method of Needleman and Wunsch (J. Mol. Biol. 48:443-453 (1970)) using the set of default parameters for pairwise comparison (for amino acid sequence comparison: Gap Creation Penalty = 8, Gap Extension Penalty = 2; for nucleotide sequence comparison: Gap Creation Penalty = 50; Gap Extension Penalty = 3).

The percentage of identity can also be calculated by using other alignment program well known in the art. For example, the percentage of identity can be calculated using the program needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 5.5 software package, using the parameters (for example GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

These above-mentioned analyses for comparing sequences may be done on full-length sequences but additionally or alternatively the calculation of sequence identity or similarity can be based on a comparison of certain regions such as conserved domains. The identification of such domains, would also be well within the realm of a person skilled in the art and involves, for example, running a computer readable format of the nucleic acids of the present invention in alignment software programs, scanning publicly available information on protein domains, conserved motifs and boxes. This type of information on protein domains is available in the PRODOM (http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html), PIR (http://pir.georgetown.edu/), INTERPRO (http://www.ebi.ac.uk/interpro/) or pFAM (http://pfam.wustl.edu/) database. Sequence analysis programs designed for motif searching can be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to: MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 2.2) can be found in version 10.0 of the GCG package; or on the Internet site http://www.sdsc.edu/MEME/meme. SIGNALSCAN version 4.0 information is available on the Internet site http://biosci.cbs.umn.edu/software/sigscan.html. GENESCAN can be found on the Internet site http://gnomic.stanford.edu/GENESCANW.html.

As mentioned above the nucleic acid suitable for practising the methods of the present invention can be wild type (native or endogenous). Alternatively, the nucleic acid may be derived from another (or the same) species, which gene is introduced into the plant as a transgene, for example by transformation. The nucleic acid may thus be derived (either directly or indirectly (if subsequently modified)) from any source provided that the nucleic acid, when expressed in a plant, leads to modified expression of a nucleic acid/gene or modified activity and/or levels of a protein essentially similar to SEQ ID NO 1 to 2755. The nucleic acid may be isolated from a microbial source, such as bacteria, yeast or fungi, or from a plant, algae, insect, or animal (including human) source. Methods for the search and identification of other homologues of the proteins of the present invention, or for nucleic acid sequences encoding homologues of proteins of the present invention would be well known to person skilled in the art. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins used in the methods according to the invention.

A preferred homologue is a homologue obtained from a plant, whether from the same plant species or different. The nucleic acid may be isolated from a dicotyledonous species, preferably from the family *Brassicaceae*, further preferably from *Arabidopsis thaliana*.

Suitable homologues for use in the methods of the present invention have been identified in the genomes of rice and maize. These homologues are represented by their Genbank accession numbers in Table 1 and 2. Other homologues, especially orthologues from other plant species, are identifiable by methods well known by a person skilled in the art. *In silico*, methods involve running sequence alignment programs with the sequence of interest as mentioned above. *In vivo* methods involve the DNA encoding the protein of interest and are for example PCR techniques using degenerated primers designed based on the sequence of interest, which is any one essentially similar to any one of SEQ ID NO 1 to 2755, or hybridisation techniques with at least part of the sequence of interest.

"Substitutional variants" of a protein are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues.

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in the protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, $(histidine)_6$-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein are characterized by the removal of one or more amino acids from the protein. Amino acid variants of a protein may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

The term "derivatives" of a protein according to the present invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise substitutions, deletions or additions of naturally and non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the protein as deposited under the accession numbers presented in Table 1, 2, 4 and 5. "Derivatives" of a protein of the present invention encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein of the present invention.

Another variant useful in the methods of the present invention is an active fragment of a protein essentially similar to any one of SEQ ID NO 1 to 2755. The expression "functional fragment" in relation to a protein refers to a fragment that encompasses contiguous amino acid residues of said protein, and that has retained the biological activity of said naturally-occurring protein. For example, useful fragments comprise at least 10 contiguous amino acid residues of a protein essentially similar to any one of SEQ ID NO 1 to 2755. Other preferred fragments are fragments of these proteins starting at the second or third or further internal methionin residues. These fragments originate from protein translation, starting at internal ATG codons.

Advantageously, the method according to the present invention may also be practiced using fragments of DNA or of a nucleic acid sequence. The term "DNA fragment or DNA segment"

be created artificially, such as for example by the techniques of EMS mutagenesis. Typically such variants are created with the purpose of breeding the altered plant characteristic according to the present invention in a plant. Alternatively, naturally mutated alleles are the subject of such selection and breeding programmes, wherein the allele capable of conferring altered plant characteristics to the plant are selected and plants containing such allele are used for further breeding the trait.

Accordingly, the present invention provides a method for altering plant characteristics, using a splice variant or an allelic variant of a nucleic acid sequence according to any one of SEQ ID NO 1 to 2755.

The term "plant characteristic" means any characteristic of a plant, plant cell or plant tissue described hereafter. These characteristics encompass but are not limited to, characteristics of plant development, plant growth, yield, biomass production, plant architecture, plant biochemistry, plant physiology, metabolism, survival capacity, stress tolerance and more. DNA synthesis, DNA modification, endoreduplication, cell cycle, cell wall biogenesis, transcription regulation, signal transduction, storage lipid mobilization, photosynthesis and more.

The term "altering plant characteristics" as used herein encompasses any change in said characteristic such as increase, decrease or change in time or place. According to a preferred embodiment of the invention, altering a plant characteristics encompasses improving the plant characteristic, such as for example increasing the plant characteristic (e.g. yield), or accelerating the characteristic (e.g. growth rate).

"Growth" refers to the capacity of the plant or of plant parts to expand and increase in biomass. Altered growth refers amongst others to altered growth rate, cycling time, the size, expansion or increase of the plant. Additionally and/or alternatively, growth characteristics may refer to cellular processes comprising, but not limited to, cell cycle (entry, progression, exit), cell division, cell wall biogenesis and/or DNA synthesis, DNA modification and/or endoreduplication.

"Yield" refers to the harvestable part of the plant. "Biomass" refers to any part of the plants. These terms also encompass an increase in seed yield, which includes an increase in the biomass of the seed (seed weight) and/or an increase in the number of (filled) seeds and/or in the size of the seeds and/or an increase in seed volume, each relative to corresponding wild-type plants. An increase in seed size and/or volume may also influence the composition of seeds. An increase in seed yield could be due to an increase in the number and/or size of

flowers. An increase in yield may also increase the harvest index, which is expressed as a ratio of the total biomass over the yield of harvestable parts, such as seeds.

"Plant development" means any cellular process of a plant that is involved in determining the developmental fate of a plant cell, in particular the specific tissue or organ type into which a progenitor cell will develop. Typical plant characteristics according to the present invention are therefore characteristics relating to cellular processes relevant to plant development such as for example, morphogenesis, photomorphogenesis, shoot development, root development, vegetative development, reproductive development, stem elongation, flowering, regulatory mechanisms involved in determining cell fate, pattern formation, differentiation, senescence, time of flowering and/or time to flower.

"Plant architecture", as used herein refers to the external appearance of a plant, including any one or more structural features or a combination of structural features thereof. Such structural features include the shape, size, number, position, colour, texture, arrangement, and patternation of any cell, tissue or organ or groups of cells, tissues or organs of a plant, including the root, stem, leaf, shoot, petiole, trichome, flower, petal, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, fruit, cambium, wood, heartwood, parenchyma, aerenchyma, sieve element, phloem or vascular tissue, amongst others.

The term "stress tolerance" is understood as the capability of better survival and/or better performing in stress conditions such as environmental stress, which can be biotic or abiotic. Salinity, drought, heat, chilling and freezing are all described as examples of conditions which induce osmotic stress. The term "environmental stress" as used in the present invention refers to any adverse effect on metabolism, growth or viability of the cell, tissue, seed, organ or whole plant which is produced by a non-living or non-biological environmental stressor. More particularly, it also encompasses environmental factors such as water stress (flooding, water logging, drought, dehydration), anaerobic (low level of oxygen, CO2 etc.), aerobic stress, osmotic stress, salt stress, temperature stress (hot/heat, cold, freezing, frost) or nutrients deprivation, pollutants stress (heavy metals, toxic chemicals), ozone, high light, pathogen (including viruses, bacteria, fungi, insects and nematodes) and combinations of these. Biotic stress is stress as a result of the impact of a living organism on the plant. Examples are stresses caused by pathogens (virus, bacteria, nematodes insects etc.). Another example is stress caused by an organism, which is not necessarily harmful to the plant, such as the stress caused by a symbiotic or an epiphyte. Accordingly, particular plant characteristics according to the present invention encompass early vigour, survival rate, stress tolerance.

Field-grown plants almost always experience some form of stress, albeit mild, and therefore the terms "growth", "yield" "biomass production" or "biomass" do not distinguish the performance of plants under non-stressed from performance under stress conditions. Advantageously, the effects of the invention on growth and yield are expected to occur under both severe and mild stress conditions (i.e. under stressed and non-stressed conditions).

Characteristics related to "plant physiology" encompass characteristics of functional processes of a plant, including developmental processes such as growth, expansion and differentiation, sexual development, sexual reproduction, seed set, seed development, grain filling, asexual reproduction, cell division, dormancy, germination, light adaptation, photosynthesis, leaf expansion, fiber production, secondary growth or wood production, amongst others; responses of a plant to externally-applied factors such as metals, chemicals, hormones, growth factors, environment and environmental stress factors (e.g. anoxia, hypoxia, high temperature, low temperature, dehydration, light, day length, flooding, salt, heavy metals, amongst others), including adaptive responses of plants to said externally-applied factors. Particular plant physiology characteristics which are altered according to the methods of the present invention encompass altered storage lipid mobilization, photosynthesis, transcription regulation and signal transduction.

Characteristics related to "plant biochemistry" are to be understood by those skilled in the art to refer to the metabolic characteristics. "Metabolism" as used in the present invention is interchangeable with biochemistry. Metabolism and/or biochemistry encompass catalytic or assimilation or other metabolic processes of a plant, including primary and secondary metabolism and the products thereof, including any element, small molecules, macromolecules or chemical compounds, such as but not limited to starches, sugars, proteins, peptides, enzymes, hormones, growth factors, nucleic acid molecules, celluloses, hemicelluloses, calloses, lectins, fibres, pigments such as anthocyanins, vitamins, minerals, micronutrients, or macronutrients, that are produced by plants. Preferably, the methods of the present invention are used to change the nitrogen or carbon metabolism.

As shown in Tables 1 and 2, several of the E2Fa-DPa target genes identified have an E2F recognition sequence in their promoter and most of these genes are involved in DNA replication. Therefore, provided by a particular embodiment of the present invention is a method as described above to influence DNA synthesis and DNA replication. The secondary induced genes, which are the genes not having the E2F target consensus sequence in their promoter region, encode proteins involved in cell wall biosynthesis, transcription, signal

transduction, or have an unknown function. Surprisingly, a large number of metabolic genes were modified as well, mainly genes involved in nitrate assimilation or metabolism and carbon metabolism.

The putative direct E2Fa-DPa target genes as identified by the presence of an E2F-DP-binding site, mainly belong to the group of genes involved in DNA synthesis, whereas the secondary induced genes are mainly linked to nitrogen assimilation and carbohydrate metabolism. Therefore, it is elucidated by the present invention that enhanced levels of E2Fa-DPa in plants have an impact on expression levels of genes involved in nitrogen assimilation and/or carbon metabolism. The experimental data suggest that in E2Fa/DPa overexpressing plants there is a drain of nitrogen to the nucleotide synthesis pathway causing a decreased synthesis of other nitrogen compounds such as amino acids and storage proteins. Corresponding to these findings, the inventors found that the level of endoreduplication of E2Fa-DPa transgenic plants depends on the amount of nitrogen available in the medium. Also, these data suggest that the growth arrest observed upon E2Fa/DPa expression results at least from a nitrogen drain to the nucleotide synthesis pathway, causing a decreased synthesis of other nitrogen components, such as amino acids and storage components.

As purine and pyrimidine bases are nitrogen-rich, the induction of nitrogen assimilation genes in the E2Fa-DPa transgenic plants is a mechanism to supply enough nitrogen for nucleotide biosynthesis. Most likely this drain of nitrogen from essential biosynthetic pathways to the nucleotide biosynthesis pathway has its effects on many aspects of plant metabolism, as can be seen from the reduction of expression of vegetative storage protein genes and genes involved in amino-acid biosynthesis.

Therefore a particular aspect of the invention is the use of genes involved in carbon and/or nitrogen metabolism or allocation, for altering nitrogen and carbon metabolism and/ or to alter the balance between carbon and nitrogen or to reallocate carbon and/or nitrogen or to alter the composition of components containing carbon and nitrogen. The elucidation of genes that are able to shift the nitrogen assimilation from one biological process to another biological process is important for many applications. These genes can for example be used to alter the nitrogen composition of nitrogen-containing compounds in a cell, such as nicotinamide-containing molecules, amino acid, nucleic acid, chlorophyll or any other metabolites. Also within the scope of the present inventions are these altered components obtainable by the methods of the present invention, with altered balance between carbon and nitrogen.

Therefore, according to the present invention, there is provided a method as described above, wherein said altered metabolism comprises altered nitrogen and/or carbon metabolism.

In a particular embodiment, said carbon metabolism comprises the processes of carbon fixation, photosynthesis and photorespiration. In another embodiment, said nitrogen metabolism comprises nitrogen fixation or the reallocation of nitrogen residues from the pool of amino acids into the pool of nucleic acids or vice versa.

Microarray analysis of E2Fa-DPa overexpressing lines, as herein described, identified a cross-talking matrix between DNA replication, nitrogen assimilation and photosynthesis. It has been described previously that there is a link between carbon:nitrogen availability and growth, storage lipid mobilization and photosynthesis (Martin T. (2002)). Therefore according to the present invention there is provided, a method as described above, wherein said altered plant characteristic comprises altered storage lipid mobilization and/or photosynthesis.

The microarray studies elucidated for the first time particular genes that are upregulated and particular genes that are downregulated in a plant cell overexpressing E2Fa/DPa, many of which were of unknown function. It is now disclosed how to use these genes and/or proteins for altering plant characteristics.

According to a preferred embodiment, recombinant means are used to alter plant characteristics. More preferably, one or more of the genes essentially similar to any of SEQ ID NO 1 to 2755 is introduced into a plant as a transgene. Accordingly, the present invention provides a recombinant nucleic acid comprising:

(a) one or more nucleic acid sequences essentially similar to any one of SEQ ID NO 1 to 2755; optionally operably linked to

(b) a regulatory sequence; and optionally operably linked to

(c) a transcription termination sequence.

This recombinant nucleic acid is suitable for altering plant growth characteristics.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.

The genetic construct can be an expression vector wherein said nucleic acid sequence is operably linked to one or more control sequences allowing expression in prokaryotic and/or eukaryotic host cells.

The methods according to the present invention may also be practised by introducing into a plant at least a part of a (natural or artificial) chromosome (such as a Bacterial Artificial Chromosome (BAC)), which chromosome contains at least a gene/nucleic acid according to the present invention, optionally together with one or more related gene family members or genes belonging to the same functional group as for example the functional groups presented in Table 1 or 2. Therefore, according to a further aspect of the present invention, there is provided a method to alter plant characteristics, comprising introduction into a plant at least a part of a chromosome comprising at least a gene/nucleic, which gene/nucleic is essentially similar to any one of SEQ ID NO 1 to 2755.

In a particular embodiment of the present invention said regulatory sequence is a plant-expressible promoter. In a further embodiment of the invention the promoter is a constitutive promoter, such as the GOS2 promoter, the ubiquitin promoter, the actin promoter. In another embodiment of the invention the promoter is a promoter active in the meristem or in dividing cells, such as, but not limited to the cdc2 promoter, RNR promoter, MCM3 promoter. Alternatively, the regulatory element as mentioned above can be a translational enhancer, or a transcriptional enhancer that is used to enhance expression of a gene according to the present invention.

The term "Regulatory sequence" refers to control DNA sequences, which are necessary to affect expression of coding sequences to which they are operably linked. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes generally control sequences include promoters, terminators and enhancers or silencers. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components and which determines when, how much and where a specific gene is expressed. Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences derived from a classical eukaryotic genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. The term "promoter" also

includes the transcriptional regulatory sequences of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or a -10 box transcriptional regulatory sequences.

The term "promoter" is also used to describe a synthetic or fusion molecule or derivative, which confers; activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. "Promoter" is a DNA sequence generally described as the 5'-region of a gene, located proximal to the start codon. The transcription of an adjacent DNA segment is initiated at the promoter region. In the context of the present invention, the promoter preferably is a plant-expressible promoter sequence. Promoters, however, that also function or solely function in non-plant cells such as bacteria, yeast cells, insect cells and animal cells are not excluded from the invention. By "plant-expressible" is meant that the promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ.

Preferably, the nucleic acid sequence capable of modulating expression of a gene encoding an E2F target protein is operably linked to a constitutive promoter or a tissue specific promoter. The term "constitutive" as defined herein refers to a promoter that is active predominantly in at least one tissue or organ and predominantly at any life stage of the plant. Preferably the promoter is active predominantly but not exclusively throughout the plant
Additionally and/or alternatively, the nucleic acid of the present invention may be operably linked to a tissue-specific promoter. The term "tissue-specific" promoter as defined herein refers to a promoter that is active predominantly but not exclusively in at least one tissue or organ.

Examples of preferred promoters useful for the methods of the present invention are presented in Table I, II, III and IV.

**Table I:**

**Exemplary constitutive promoters for use in the performance of the present invention**

| GENE SOURCE | EXPRESSION PATTERN | REFERENCE |
|---|---|---|
| Actin | constitutive | McElroy *et al,* Plant Cell, 2: 163-171, 1990 |
| CAMV 35S | constitutive | Odell *et al,* Nature, 313: 810-812, 1985 |
| CaMV 19S | constitutive | Nilsson *et al., Physiol. Plant. 100*:456-462, 1997 |
| GOS2 | constitutive | de Pater *et al,* Plant J Nov;2(6):837-44, 1992 |
| ubiquitin | constitutive | Christensen *et al,* Plant Mol. Biol. 18: 675-689, 1992 |

| | | |
|---|---|---|
| rice cyclophilin | constitutive | Buchholz *et al*, Plant Mol Biol. 25(5): 837-43, 1994 |
| maize H3 histone | constitutive | Lepetit *et al*, Mol. Gen. Genet. 231:276-285, 1992 |
| actin 2 | constitutive | An *et al*, Plant J. 10(1); 107-121, 1996 |

**Table II:**

**Exemplary seed-preferred promoters for use in the performance of the present invention**

| GENE SOURCE | EXPRESSION PATTERN | REFERENCE |
|---|---|---|
| seed-specific genes | seed | Simon, *et al., Plant Mol. Biol. 5:* 191, 1985; Scofield, *et al., J. Biol. Chem. 262:* 12202, 1987.; Baszczynski, *et al., Plant Mol. Biol. 14:* 633, 1990. |
| Brazil Nut albumin | seed | Pearson, *et al.*, Plant Mol. Biol. 18: 235-245, 1992. |
| legumin | seed | Ellis, *et al.*, Plant Mol. Biol. 10: 203-214, 1988. |
| glutelin (rice) | seed | Takaiwa, *et al.*, Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, *et al.*, FEBS Letts. 221: 43-47, 1987. |
| zein | seed | Matzke et al Plant Mol Biol, 14(3):323-32 1990 |
| napA | seed | Stalberg, *et al, Planta 199:* 515-519, 1996. |
| wheat LMW and HMW glutenin-1 | endosperm | Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989 |
| wheat SPA | seed | Albani *et al*, Plant Cell, 9: 171-184, 1997 |
| wheat a, b and g-gliadins | endosperm | EMBO 3:1409-15, 1984 |
| barley *ltr1* promoter | endosperm | |
| barley B1, C, D, hordein | endosperm | Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996 |
| barley DOF | endosperm | Mena *et al*, The Plant Journal, 116(1): 53-62, 1998 |
| *blz2* | endosperm | EP99106056.7 |
| synthetic promoter | endosperm | Vicente-Carbajosa *et al., Plant J. 13:* 629-640, 1998. |
| rice prolamin NRP33 | endosperm | Wu *et al*, Plant Cell Physiology 39(8) 885-889, 1998 |
| rice -globulin Glb-1 | endosperm | Wu *et al*, Plant Cell Physiology 39(8) 885-889, 1998 |
| rice OSH1 | embryo | Sato *et al*, Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996 |
| rice alpha-globulin REB/OHP-1 | endosperm | Nakase *et al.* Plant Mol. Biol. 33: 513-522, 1997 |
| rice ADP-glucose PP | endosperm | Trans Res 6:157-68, 1997 |
| maize ESR gene family | endosperm | Plant J 12:235-46, 1997 |
| sorgum gamma-kafirin | endosperm | PMB 32:1029-35, 1996 |

| | | |
|---|---|---|
| KNOX | embryo | Postma-Haarsma *et al*, Plant Mol. Biol. 39:257-71, 1999 |
| rice oleosin | embryo and aleuron | Wu *et at*, J. Biochem., 123:386, 1998 |
| sunflower oleosin | seed (embryo and dry seed) | Cummins, *et al.*, Plant Mol. Biol. 19: 873-876, 1992 |

**Table III:**

**Exemplary flower-specific promoters for use in the performance of the invention**

| Gene source | Expression pattern | REFERENCE |
|---|---|---|
| AtPRP4 | flowers | http://salus.medium.edu/mmg/tierney/html |
| chalene synthase (chsA) | flowers | Van der Meer, *et al., Plant Mol. Biol. 15*, 95-109, 1990. |
| LAT52 | anther | Twell *et al* Mol. Gen Genet. 217:240-245 (1989) |
| *apetala-3* | flowers | |

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**Table IV:**

**Alternative rice promoters for use in the performance of the invention**

| PRO # | gene | expression |
|---|---|---|
| | | |
| PRO0001 | Metallothionein Mte | transfer layer of embryo + calli |
| PRO0005 | putative beta-amylase | transfer layer of embryo |
| PRO0009 | putative cellulose synthase | weak in roots |
| PRO0012 | lipase (putative) | |
| PRO0014 | transferase (putative) | |
| PRO0016 | peptidyl prolyl cis-trans isomerase (putative) | |
| PRO0019 | unknown | |
| PRO0020 | prp protein (putative) | |
| PRO0029 | noduline (putative) | |
| PRO0058 | proteinase inhibitor Rgpi9 | seed |
| PRO0061 | beta expansine EXPB9 | weak in young flowers |
| PRO0063 | structural protein | young tissues+calli+embryo |
| PRO0069 | xylosidase (putative) | |
| PRO0075 | prolamine 10 Kda | strong in endosperm |
| PRO0076 | allergen RA2 | strong in endosperm |
| PRO0077 | prolamine RP7 | strong in endosperm |
| PRO0078 | CBP80 | |
| PRO0079 | starch branching enzyme I | |
| PRO0080 | Metallothioneine-like ML2 | transfer layer of embryo + calli |
| PRO0081 | putative caffeoyl-CoA 3-O-methyltransferase | shoot |
| PRO0087 | prolamine RM9 | strong in endosperm |
| PRO0090 | prolamine RP6 | strong endosperm |
| PRO0091 | prolamine RP5 | strong in endosperm |
| PRO0092 | allergen RA5 | |
| PRO0095 | putative methionine aminopeptidase | embryo |
| PRO0098 | ras-related GTP binding protein | |
| PRO0104 | beta expansine EXPB1 | |
| PRO0105 | Glycine rich protein | |
| PRO0108 | metallothionein like protein (putative) | |
| PRO0109 | metallothioneine (putative) | |
| PRO0110 | RCc3 | strong root |
| PRO0111 | uclacyanin 3-like protein | weak discrimination center / shoot meristem |
| PRO0116 | 26S proteasome regulatory particle non-ATPase subunit 11 | very weak meristem specific |

| | | |
|---|---|---|
| PRO0117 | putative 40S ribosomal protein | weak in endosperm |
| PRO0122 | chlorophyll a/b-binding protein precursor (Cab27) | very weak in shoot |
| PRO0123 | putative protochlorophyllide reductase | strong leaves |
| PRO0126 | metallothionein RiCMT | strong discrimination center / shoot meristem |
| PRO0129 | GOS2 | strong constitutive |
| PRO0131 | GOS9 | |
| PRO0133 | chitinase Cht-3 | very weak meristem specific |
| PRO0135 | alpha-globulin | strong in endosperm |
| PRO0136 | alanine aminotransferase | weak in endosperm |
| PRO0138 | cyclin A2 | |
| PRO0139 | Cyclin D2 | |
| PRO0140 | Cyclin D3 | |
| PRO0141 | cyclophyllin 2 | shoot and seed |
| PRO0146 | sucrose synthase SS1 (barley) | medium constitutive |
| PRO0147 | trypsin inhibitor ITR1 (barley) | weak in endosperm |
| PRO0149 | ubiquitine 2 with intron | strong constitutive |
| PRO0151 | WSI18 | embryo + stress |
| PRO0156 | HVA22 homologue (putative) | |
| PRO0157 | EL2 | |
| PRO0169 | aquaporine | medium constitutive in young plants |
| PRO0170 | High mobility group protein | strong constitutive |
| PRO0171 | reversibly glycosylated protein RGP1 | weak constitutive |
| PRO0173 | cytosolic MDH | shoot |
| PRO0175 | RAB21 | embryo + stress |
| PRO0176 | CDPK7 | |
| PRO0177 | Cdc2-1 | very weak in meristem |
| PRO0197 | sucrose synthase 3 | |
| PRO0198 | OsVP1 | |
| PRO0200 | OSH1 | very weak in young plant meristem |
| PRO0208 | putative chlorophyllase | |
| PRO0210 | OsNRT1 | |
| PRO0211 | EXP3 | |
| PRO0216 | phosphate transporter OjPT1 | |
| PRO0218 | oleosin 18kd | aleurone + embryo |
| PRO0219 | ubiquitine 2 without intron | |
| PRO0220 | RFL | |

| | | |
|---|---|---|
| PRO0221 | maize UBI delta intron | not detected |
| PRO0223 | glutelin-1 | |
| PRO0224 | fragment of prolamin RP6 promoter | |
| PRO0225 | 4xABRE | |
| PRO0226 | glutelin OSGLUA3 | |
| PRO0227 | BLZ-2_short (barley) | |
| PRO0228 | BLZ-2_long (barley) | |

Optionally, one or more terminator sequences may also be used in the construct introduced into a plant. The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences, which may be suitable for use in performing the invention. Such sequences would be known or may readily be obtained by a person skilled in the art.

The genetic constructs of the invention may further include an origin of replication sequence which is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

The genetic construct may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" includes any gene, which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a nucleic acid construct of the invention. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance, that introduce a new metabolic trait or that allow visual selection. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as nptII encoding neomycin phosphotransferase capable of phosphorylating neomycin and kanamycin, or hpt encoding hygromycin phosphotransferase capable of phosphorylating hygromycin), to herbicides (for example bar which provides resistance to Basta; aroA or gox providing resistance against glyphosate), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source). Visual marker genes result in the formation of colour (for example beta-glucuronidase, GUS), luminescence (such as luciferase) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). Further examples of suitable selectable marker genes include the ampicillin resistance (Ampr), tetracycline resistance gene (Tcr),

bacterial kanamycin resistance gene (Kanr), phosphinothricin resistance gene, and the chloramphenicol acetyltransferase (CAT) gene, amongst others

The methods of the present invention are particularly relevant for applications in agriculture and horticulture, and serve to develop plants that have altered characteristics.

Accordingly, another embodiment of the invention is a method for making a transgenic plant comprising the introduction of a recombinant nucleic acid as mentioned above into a plant. "A plant" as used herein means plant cell, plant part etc. as defined herein below. According to a preferred embodiment this method for the production of a transgenic plant further comprises the step of cultivating the plant cell under conditions promoting regeneration and mature plant growth.

A further embodiment relates to a method as described above, comprising stably integrating into the genome of a plant a recombinant nucleic acid as mentioned above. Alternatively, the recombinant nucleic acids comprising the nucleic acids of the present invention are transiently introduced into a plant or plant cell. The protein itself and/or the nucleic acid itself may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of the plant). According to a preferred feature of the present invention, the nucleic acid is preferably introduced into a plant by transformation.

The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g. cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively and preferably, the transgene may be stably integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable

ancestor cell. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens *et al.*, 1982; Negrutiu *et al.*, 1987); electroporation of protoplasts (Shillito *et al.*, 1985); microinjection into plant material (Crossway *et al.*, 1986); DNA or RNA-coated particle bombardment (Klein *et al.*, 1987) infection with (non-integrative) viruses and the like.

Transgenic rice plants expressing a gene according to the present invention are preferably produced via *Agrobacterium*-mediated transformation using any of the well known methods for rice transformation, such as described in any of the following: published European patent application EP 1198985 A1, Aldemita and Hodges (1996); Chan *et al.* (1993), Hiei *et al.* (1994), which disclosures are incorporated by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida *et al.* (1996) or Frame *et al.* (2002), which disclosures are incorporated by reference herein as if fully set forth.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

The present invention also encompasses plants obtainable by the methods according to the present invention. The present invention provides plants having one or more altered characteristics, when compared to the wild-type plants, characterised in that the plant has modified expression of one or more nucleic acids and/or modified level and/or activity of a protein, wherein said nucleic acid and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755.

In one embodiment of the present invention, such a plant is a transgenic plant. According to a further embodiment such transgenic plant comprises an isolated nucleic acid and/or protein sequence essentially similar to any one for Seq Id NO 1 to 2755.

Alternatively, according to one embodiment of the present invention, such a plant having one or more altered plant characteristics and having modified expression of one or more nucleic acids and/or modified level and/or activity of a protein, wherein said nucleic acid and/or protein are essentially similar to any one of SEQ ID NO 1 to 2755., is created by breeding techniques.

The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced in the parent by the methods according to the invention. The invention accordingly also includes host cells containing an isolated nucleic acid molecule encoding a protein essentially similar to any one of SEQ ID NO 1 to 2755. Such host cell may be selected from plants, bacteria, animals, algae, fungi, yeast or insects. Preferred host cells according to the invention are plant cells. The invention also extends to harvestable parts of a plant such as but not limited to seeds, leaves, fruits, flowers, stem cultures, stem, rhizomes, roots, tubers and bulbs.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant" also therefore encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp.,*

*Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays,* amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, trees. Alternatively algae and other non-Viridiplantae can be used for the methods of the present invention. Preferably the plant according to the present invention is a crop plant selected from rice, maize, wheat, barley, soybean, sunflower, canola, sugarcane, alfalfa, millet, leguminosae (bean, pea), flax , lupinus, rapeseed, tobacco, popular and cotton. Further preferably, the plant according to the present invention is a monocotyledonous plant, most preferably a cereal.

The term 'gene(s)' or 'nucleic acid', 'nucleotide sequence', as used herein refers to a polymeric form of a deoxyribonucleotides or ribonucleotide polymer of any length, either double- or single-stranded, or analogs thereof, that have the essential characteristics of a natural ribonucleotide in that they can hybridize to nucleic acids in a manner similar to naturally occurring polynucleotides. A great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those skilled in the art. For example, methylation, 'caps' and substitution of one or more of the naturally occurring nucleotides with an analog. Said terms also include peptide nucleic acids. The term "polynucleotide" as used herein includes such chemically, enzymatyically or metabolically modified forms of polynucleotides.

With "recombinant nucleic acid" is meant a nucleic acid produced by joining pieces of DNA from different sources through deliberate human manipulation.

The inventors identified genes that are upregulated in plants overexpressing E2Fa/DPa. These genes can be used to simulate E2Fa/DPa related effect in a plant.

Therefore, according to the invention, there is provided a method to alter characteristics of a plant, comprising overexpression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755, or wherein the method comprises enhancing the level and/or activity of one or more proteins essentially similar to a protein sequence essentially similar to any one of SEQ ID NO 1 to 2755.

Also identified were genes that are downregulated in plants overexpressing E2Fa/DPa. These genes can be used to simulate E2Fa/DPa related effect in a plant. Therefore, according to the invention, there is provided a method to alter plant growth characteristics, comprising downregulation of expression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755, or wherein the method comprises decreasing level and/or activity of one or more proteins essentially similar to any one of SEQ ID NO 1 to 2755.

Genetic constructs aimed at silencing gene expression may comprise the nucleotide sequence essentially similar to any one of SEQ ID NO 1 to 2755 or one at least a portion thereof in a sense and/or antisense orientation relative to the promoter sequence. Preferably the portions comprises at least 21 contiguous nucleic acid of a sequence to be downregulated. Also, sense or antisense copies of at least part of the endogenous gene in the form of direct or inverted repeats may be utilized in the methods according to the invention. The characteristics of plants may also be changed by introducing into a plant at least part of an antisense version of the nucleotide sequence essentially similar to any one or more of SEQ ID NO 1 to 2755. It should

be clear that part of the nucleic acid (a portion) could also achieve the desired result. Homologous anti-sense genes are preferred, homologous genes being plant genes, preferably plant genes from the same plant species in which the silencing construct is introduced.

Detailed analysis of the promoters of the genes identified in the present invention allowed the identification of novel E2Fa/DPa target genes that are under the direct control of E2Fa/DPa and that are mainly involved in DNA replication. For all the genes identified in the present invention, reference is made to the MIPS database MATDB accession number. This unique identification number refers to the deposit of information related to the gene in question, e.g. the unspliced sequence, the spliced sequence, the protein sequence, the domains of the protein etc. An example of the information deposited under the accession number At1g57680 is shown in Figure 4. Based on this unique accession number, a person skilled in the art would be able to locate the gene provided by the present invention in its genomic environment. From this information one can identify and isolate the upstream control elements of these genes. Especially interesting are the promoters of these genes as control elements for driving or regulating transcription of heterologous genes. Therefore, according to the invention is provided an isolated nucleic acid comprising one or more of the regulatory elements upstream of the startcodon of the nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755. Furthermore, the invention provides an isolated nucleic acid as mentioned above, wherein said regulatory element is the promoter of said the genes essentially similar to any one of the sequence presented in SEQ ID NO 1 to 2755.

Further the invention also relates to the use of a nucleic acid sequence or protein essentially similar to any one of SEQ ID NO 1 to 2755, for altering plant characteristics.

Another method for altering plant characteristics and/or growth characteristics of a plant resides in the use of allelic variants of the genes of the present invention. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these natural alleles. Alternatively, in particular breeding programs, such as for example marker assisted breeding, or conventional breeding programmes, it is sometimes practical to introduce allelic variation in the plants by mutagenic treatment of a plant. One suitable mutagenic method is EMS mutagenesis. Identification of allelic variants then takes place by, for example, PCR. This is followed by a selection step for selection of superior allelic variants of the sequence in question and which give rise to altered growth characteristics. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question. Monitoring growth performance can be done in a greenhouse or in the field. Further optional steps include crossing plants in which the superior

allelic variant was identified with another plant. This could be used, for example, to make a combination of interesting phenotypic features.

According to another aspect of the present invention, advantage may be taken of the nucleic acid sequence of the present invention in breeding programs. In such a program, a DNA marker may be identified which is genetically linked to the nucleic acid of the present invention. This DNA marker is then used in breeding programs to select plants having altered growth characteristics. Therefore, the present invention also encompass the use of a nucleic acid sequence essentially similar to any one of SEQ ID NO 1 to 2755, for marker assisted breeding of plants with altered characteristics.

These marker assisted breeding processes may further involve the steps of crossing plants and using probes or primers having part, for example having at least 10 bp, of a sequence corresponding to any of SEQ ID NO 1 to 2755, to detect the DNA sequence corresponding to SEQ ID NO 1 to 2755, in the progeny of the cross.

These methods for marker assisted breeding also may involve the use of an isolated DNA molecule being essentially similar to SEQ ID NO 1 to 2755 or a part thereof as a marker in techniques like AFLP, RFLP, RAPD, or in the detection of Single Nucleotide Polymorphisms.

Further these methods for marker assisted breeding also may involve determining the presence or absence in a plant genome of a qualitative trait or a quantitative trait locus (QTL) linked to a transgene essentially similar to any one of SEQ ID NO 1 to 2755 or to an endogenous homologue of any one of SEQ ID NO 1 to 2755, which method comprises:

(a) detecting a molecular marker linked to a QTL, wherein the molecular marker comprises a sequence essentially similar to SEQ ID NO 1 to 2755or an endogenous homologue thereof; and

(b) determining the presence of said QTL as by detection of the molecular marker of step (a) or determining the absence of said QTL as failure to detect the molecular marker of step (a)

Alternatively, methods for marker assisted breeding may involve detecting the presence of a quantitative trait locus linked to a DNA sequence essentially similar to SEQ ID NO 1 to 2755 or to an endogenous homologue thereof in the genome of a plant. The methods described above may involve the steps of:

(a) extracting a DNA sample of said plant;

(b) contacting the DNA sample with a probe that hybridises to a DNA sequence according to claim 1 or to an endogenous homologue thereof, or to the complement thereof;

(c) performing a hybridisation reaction under conditions suitable for hybridisation of the probe to the DNA sample of (b); and

(d) detecting the hybridisation of the probe to the DNA.

Further, the present invention also encompass the use of a nucleic acid sequence essentially similar to any one of SEQ ID NO 1 to 2755, for conventional breeding of plants with altered characteristics.

In conventional breeding programs, the nucleic acid essentially similar to any one of SEQ ID NO 1 to 2755 is used to select plants with better plant characteristics compared to the normal wild-type plants. The plants with better growth characteristics may originated from natural variation in the alleles of the gene corresponding to any one of SEQ ID NO 1 to 2755, or may originated from manmade variation in these genes, for example variation created by EMS mutagenesis or other methods to created single nucleotide polymorfisms.

Further the invention also relates to the use of a nucleic acid or a protein essentially similar any one of SEQ ID NO 1 to 2755, as a growth regulator.

In a particular embodiment such a growth regulator is a herbicide or is a growth stimulator. The present invention therefore also provides a growth regulating composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755. The growth regulating compositions according to the present invention can additionally comprise any additive usually present in growth regulating compositions such as growth inhibitors, herbicides or growth stimulators. Also a kit comprising a sequence essentially similar to any one of SEQ ID NO 1 to 2755 (for example in the form of a herbicide) is in the scope of the present invention. Also any other plant effective agent comprising the sequences according to the present invention are provided herein. Methods to produce the compositions, kits or plant agents as mentioned above are also provided by the present invention and involve the production of any one or more of the sequences essentially similar to any one of SEQ ID NO 1 to 2755. Such sequences and methods are herein provided.

Further, plants of the present invention have improved characteristics, such as improved growth and yield, which makes these plant suitable to produce industrial proteins.

Accordingly, the present invention provides a method for the production of enzymes and/or pharmaceuticals, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755

The present invention therefore also encompasses the use of plants as described above, for the production of (industrial) enzymes and/or pharmaceuticals. The (Industrial) enzymes and pharmaceuticals produced according to the method as described above are also encompasses by the present invention.

Also the invention as presented herein offers means to alter the characteristics not only of plants, but also of other organisms, such as mammals. The plant genes of the present invention, or their homologues, or the plant proteins or their homologues, can be used as therapeutics or can be used to develop therapeutics for both humans and animals. Accordingly, the present invention relates to a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 , for use as a therapeutic agent.

In a particular embodiment, the use as a therapeutic agents encompasses the use in gene therapy, or the use to manufacture medicaments such as for example therapeutic protein samples. Also the nucleic acids and/or proteins according to the present invention can be applied in diagnostic methods.

Accordingly provided by the present invention is the use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755, for use as a therapeutic agent, a diagnostic means, a kit or plant effective agent.

Further encompassed by the invention are therapeutic or diagnostic compositions or kits or plant effective agent, comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755. These compositions may comprise other additives usually applied for therapeutic compositions. Methods to produce these therapeutic or diagnostic compositions or kts are also provided by the present invention and involve the production of any of the sequences essentially similar to any one of SEQ ID NO 1 to 2755.

The plants according to the present invention have altered characteristics, such as for example improved growth and yield, which makes them suitable sources for many agricultural applications and the food industry. Accordingly, provided by the present invention there is a food product derived from a plant or host cell as described above and also the use of such a food product in animal feed or food.

In molecular biology it is standard practice to select upon transfection or transformation those individuals (or groups of individuals, such as bacterial or yeast colonies or phage plaques or eukaryotic cell clones) that are effectively transfected or transformed with the desired genetic

construct. Typically these selection procedures are based on the presence of a selectable or screenable marker in the transfected genetic construct, to distinguish the positive individuals easily from the negative individuals. The nucleic acids and proteins according to the present invention are capable of altering the characteristics of the host cells to which they are applied. Therefore, the nucleic acids and/or proteins according to the present invention can also be used as selectable markers, screenable markers or selection agents. According to one particular embodiment, the present invention provides the use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a positive or negative selectable marker during transformation of plant cell, plant tissue or plant procedures.

## DESCRIPTION OF THE FIGURES

**Figure 1:** Volcano plot of significance against effect. Each x represent one of the 4579 genes, with the negative log10 of the P value from the gene model plotted against the difference between least-square means for the genotype effect. The horizontal line represents the test-wise threshold of P=0.05. The two vertical reference lines indicate a 2-fold cutoff for either repression or induction.

**Figure 2:** Sources of alpha- ketoglutarate and other metabolites in plants, with annotation of up and downregulated genes in the E2Fa-DPa overproducing cells. Upregulated enzymes are underlined with a dashed line and enzymes underlined with a full line are downregulated in the E2Fa-DPa versus wild type plants. Products that are boxed act as precursors for nucleotide biosynthesis. A -KG, alpha-ketoglutarate; GOGAT, glutamate synthetase; NIA2, nitrate reductase, NiR, nitrite reductase.

**Figure 3:** Endoreduplication levels in wild type and E2Fa/DPa transgenic lines in relation to nitrogen availability. Wild type (A) and transgenic (B) lines were grown on minimal medium in the presence of 0.1, 1, 10, or 50 mM ammonium nitrate. Values are means of three independent measurements.

**Figure 4:** Represents the information which is deposited in the MatDB (MIPS *Arabidopsis* database) under accession number At1g57680

**Figure 5**: Verification of microarray analysis by RT-PCR. RT-PCR analysis was carried out under linear amplification conditions. The actin 2 gene (ACT2) was used as loading control. GS, glutamine synthetase; GOGAT, glutamate synthase; NiR, nitrite reductase.

**Figure 6:** NMR spectrum of E2Fa/DPa overexpressing plant cells.

**Table 1:** Presentation of *Arabidopsis* genes that are 2 fold or more upregulated in E2Fa-DPa overexpressing plants. The genes are presented according to the functional category to which they belong. For some of the genes, no function has been described in the public databases and they are named unknown, putative or hypothetical protein. All the genes have each a unique MIPS accession number, which refers to the identification of the sequence in the MatDB (MIPS *Arabidopsis thaliana* database). The MIPS accession number refers to the protein entry code for the MatDB of MIPS. Also, there is an accession number provided as an internal protein code. The fold of induction is also given for each sequence. Furthermore, where an E2F target sequence has been identified in the upstream region of the gene, the sequence of that site is also presented in the Table. Finally, other plant homologues which have substantial sequence identity with the *Arabidopsis* gene are mentioned in this Table.

**Table 2:** Presentation of *Arabidopsis* genes that are 2 fold or more repressed in E2Fa-DPa overexpressing plants. Data are presented in a similar way as for Table 1, as explained above.

**Table 3:** Different E2F target sequences and the frequency of their presence in the upstream regions of the *Arabidopsis* genes described in the present invention.

**Table 4:** Selection of the *Arabidopsis* genes from the microarray that were 1.3 times upregulated in E2Fa/DPa overexpressing plants, compared to the wild–type plants. The gene name is given, as well as the MIPS database accession number and a ratio indicating the degree of upregulation of the gene. Furthermore, the E-value indicates if a significant homologue has been found in the public databases.

**Table 5:** Selection of the *Arabidopsis* genes from the microarray that were 1.3 times repressed in E2Fa/DPa overexpressing plants, compared to the wild –type plants. The data are presented as in Table 4. The fold repression is calculated as 1/ratio. In this Table only the genes that have a ratio of less than 0.77 are selected.

**Table 6:** genes selected for Arabidopsis transformation

**Table 7:** genes selected for rice transformation

## EXAMPLES

### *Example 1. Overexpression of E2Fa and DPa in Arabidopsis*

Double transgenic CaMV35S-E2Fa-DPa overexpressing plants were obtained by the crossing of homozygous CaMV35S-E2Fa and CaMV35S-DPa plants (De Veylder et al., 2002). Double transformants were grown under a 16h light/ 8h dark photoperiod at 22°C on germination medium (Valvekens et al., 1988).

***Selection of transgenic lines***

*Arabidopsis thaliana* plants were generated that contained either the *E2Fa* or the *DPa* gene under the control of the constitutive cauliflower mosaic virus (*CaMV*) 35S promoter.

***Crossing experiments of overexpressing E2Fa and DPa lines***

Plants homozygous for the *CaMV 35S E2Fa* gene were crossed with heterozygous *CaMV 35S DPa* lines. Polymerase chain reaction (PCR) analysis on individual plants confirmed which plants contained both the *CaMV 35S-E2Fa* and *CaMV 35S-DPa* constructs.
8 days after sowing, these plants were used to isolate total RNA, from which cDNA was synthesized and subsequently hybridized to a microarray containing 4579 unique *Arabidopsis* ESTs. These experimental steps are described in the following examples.

### *Example 2: Construction of Microarrays*

***Construction of Microarrays***

The *Arabidopsis* thaliana microarray consisted of 4,608 cDNA fragments spotted in duplicate, distant from each other, on Type V silane coated slides (Amersham BioSciences, Buckinghamshire, UK). The clone set included 4,579 *Arabidopsis* genes composed from the unigen clone collection from Incyte (*Arabidopsis* Gem I, Incyte, USA). To retrieve the functional annotation of the genes relating to the spotted ESTs, BLASTN against genomic sequences was performed. To make the analysis easier a collection of genomic sequences bearing only one gene was built according to the available annotations. Each of those sequences had its upstream intergenic sequence followed by the exon-intron structure of the gene and the downstream intergenic sequence, intergenic being the whole genomic sequence between start and stop codons from neighboring protein-encoding genes. From the BLASTN output the best hits were extracted and submitted to a BLASTX search against protein databases. To retrieve even more detailed information concerning the potential function of the genes, protein domains were searched using ProDom. The complete data set can be found on the website http://www.psb.rug.ac.be/E2F and is cited herein by reference. The cDNA inserts were PCR amplified using M13 primers, purified with MultiScreen-PCR plate (cat: MANU03050, Millipore,

Belgium) and arrayed on the slides using a Molecular Dynamics Generation III printer (Amersham BioSciences). Slides were blocked in 3.5%SSC, 0.2%SDS, 1% BSA for 10 minutes at 60°C.

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***RNA amplification and labeling***

Antisense RNA amplification was performed using a modified protocol of *in vitro* transcription as described earlier in Puskas et al. (2002). For the first strand cDNA synthesis, 5 µg of total RNA was mixed with 2 µg of an HPLC-purified anchored oligo-dT + T7 promoter (5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG-$T_{24}$(A/C/G)-3`) (SEQ ID NO 2756). (Eurogentec, Belgium), 40 units of RNAseOUT (cat# 10777-019, Invitrogen, Merelbeke, Belgium) and 0.9M D(+) trehalose (cat# T-5251, Sigma Belgium) in a total volume of 11µl, and heated to 75°C for 5 minutes. To this mixture, 4 µl 5x first strand buffer (Invitrogen, Belgium), 2 l 0.1 M DTT, 1 µl 10 mM dNTP mix, 1 µl 1.7 M D(+)trehalose (cat# T-5251, Sigma Belgium) and 1 µl, 200 Units of SuperScript II (cat#: 18064-014, Invitrogen, Belgium) was added in 20 µl final volume. The sample was incubated in a Biometra-UnoII thermocycler at 37°C for 5 minutes, 45°C for 10 minutes, 10 cycles at 60°C for 2 minutes and at 55°C for 2 minutes. To the first strand reaction mix, 103.8 µl water, 33.4 µl 5x second strand synthesis buffer (Invitrogen, Belgium), 3.4 µl 10 mM dNTP mix, 1 µl of 10U/µl E.coli DNA ligase (cat#: 18052-019, Invitrogen, Belgium), 4 µl 10 U/µl E.coli DNA Polymerase I (cat#: 18010-025, Invitrogen, Belgium) and 1 µl 2U/µl E.coli RNAse H (cat#: 18021-071, Invitrogen, Belgium) was added, and incubated at 16°C for 2 hours. The synthesized double-stranded cDNA was purified with Qiaquick (cat#: 28106, Qiagen, Hilden, Germany). Antisense RNA synthesis was done by AmpliScribe T7 high yield transcription kit (cat#: AS2607; Epicentre Technologies, USA) in total volume of 20 µl according to the manufacturer's instructions. The RNA was purified with RNeasy purification kit (cat#: 74106; Qiagen, Germany). From this aRNA, 5 µg was labeled by reverse transcription using random nonamer primers (Genset, Paris, France), 0.1 mM d(G/T/A)TPs, 0.05 mM dCTP (Amersham BioSciences, UK), 0.05 mM Cy3-dCTP or Cy5-dCTP (cat#: PA53023, PA55023; Amersham BioSciences, UK) 1x first strand buffer, 10 mM DTT and 200 Units of SuperScript II (cat#: 18064-014, Invitrogen, Belgium) in 20 µl total volume. The RNA and primers were denatured at 75°C for 5 minutes and cooled on ice before adding the remaining reaction components. After 2 hours incubation at 42°C, mRNA was hydrolyzed in 250 mM NaOH for 15 minutes at 37°C. The sample was neutralized with 10 µl of 2 M MOPS and purified with Qiaquick (cat#: 28106, Qiagen, Germany).

***Array hybridization and post-hybridization processes***

The probes were resuspended in 30 µl hybridization solution (50 % formamide, 5x SSC, 0.1 % SDS, 100 mg/ml salmon sperm DNA) and prehybridized with 1µl poly-dT (1mg/ml) at 42°C for 30 minutes to block hybridization on the polyA/T tails of the cDNA on the arrays. 1 mg/ml mouse COT DNA (cat#: 18440-016, Invitrogen, Belgium) was added to the mixture and placed on the array under a glass coverslip. Slides were incubated for 18 hours at 42°C in a humid hybridization cabinet (cat#: RPK0176; Amersham BioSciences, UK). Post-hybridization

washing was performed for 10 minutes at 56°C in 1xSSC, 0.1% SDS, two times for 10 minutes at 56°C in 0.1xSSC, 0.1% SDS and for 2 minutes at 37°C in 0.1xSSC.

***Scanning and data analysis***

Arrays were scanned at 532 nm and 635 nm using a Generation III scanner (Amersham BioSciences, UK). Image analysis was performed with ArrayVision (Imaging Research Inc, Ontario, Canada). Spot intensities were measured as artifact removed total intensities (ARVol). No background correction was performed. First, within-slide normalization was addressed by plotting for each single slide a "MA-plot" (Yang et al., 2002), where $M = \log_2 (R/G)$ and $A = \log_2 0.5\sqrt{R \times G}$. The "LOWESS" normalization was applied to correct for dye-intensity differences. Subsequently, in order to normalize between slides and to identify differentially expressed genes between the two genotypes, two sequential analyses of variance (ANOVAs) were applied, proposed by Wolfinger et al. (2002), as follows: 1) firstly, the base-2 logarithm of the "LOWESS"-transformed measurements for all 73,264 spots ($y_{gklm}$) was subjected to a normalization model of the form $y_{iklm} = \mu + A_k + A_k D_l R_m + \varepsilon_{iklm}$, where $\mu$ is the sample mean, $A_k$ is the effect of the $k$th array ($k$ = 1-4), $A_k D_l R_m$ is the channel-effect (AD) for the $m$th replication of the total collection of cDNA fragments ($m$ = 2; left or right), and $\varepsilon_{iklm}$ is the stochastic error; 2) secondly, the residuals from this model were subjected to 4,579 gene-specific models of the form $r_{ijkl} = \mu + G_i A_k + G_i D_l + G_i C_j + \gamma_{ijkl}$ where $G_i A_k$ is the spot effect, $G_i D_l$ is the gene-specific dye effect, $G_i C_j$ is representing the signal intensity for genes that can specifically be attributed to the genotypes (effect of interest), and $\gamma_{ijkl}$ is the stochastic error. All effects were assumed to be fixed effects, except $\varepsilon_{klm}$ and $\gamma_{ijkl}$. A t-test for differences between the $G_i C_j$ effects was performed, where the t-tests are all based on $n_1+n_2-2$ degrees of freedom corresponding to the $n_1$ WT hybridisations and $n_2$ E2Fa-DPa hybridisations. The p-value cutoff was set at 0.01. No further adjustment for multiple testing was performed, as Bonferroni adjustment for 4,579 tests, to assure an experiment-wise false positive rate of 0.05, results in a p-value cutoff of $1e^{-5.0}$, which is certainly too conservative; therefore it was chosen to set the p-value cutoff arbitrarily at the 0.01 level. Also $G_i D_l$ effects were estimated and t-tested for significance at the 1% level in a same way as described above. Genes with a significant $G_i D_l$ effect were discarded. Genstat was used to perform both the normalization and gene model fits.

***Example 3: Results of the Microarray analysis and statistical analysis***

A micro-array containing in duplicate 4579 unique *Arabidopsis* ESTs, representing about a sixth of the total genome, was used to compare the transcriptome of wild type with that of *E2Fa-DPa* overexpressing plants. cDNA was synthesized from total RNA isolated from wild type and transgenic plants harvested 8 days after sowing. At this stage, transgenic plants were distinguished from control plants by the appearance of curled cotelydons which display ectopic

cell divisions and enhanced endoreduplication (De Veylder et al., 2002). In the first two hybridizations Cy3 and Cy5 fluorescently labeled probe pairs of control and *E2Fa-DPa* cDNA were used using independent mRNA extractions of the *E2Fa-DPa* plants. Subsequently, a dye-swap replication was performed for both hybridizations, resulting in a total of four cDNA microarray hybridizations.

Fluorescence levels were analyzed with the aim to establish whether the level of expression of each gene varied according to overexpression of the *E2Fa-DPa* transcription factor. Two sequential analyses of variance (ANOVAs) were used, as proposed by Wolfinger et al. (2002). The first ANOVA model, called the "normalization" model, accounts for experiment-wise systematic effects, such as array- and channel-effects, that could bias inferences made on the data from the individual genes. The residuals from this model represent normalized values and are the input data for the second ANOVA model, called the "gene" model. The gene models are fit separately to the normalized data from each gene. This procedure uses differences in normalized expression levels, rather than ratios, as the unit of analysis of expression differences.

Prior to the estimation of genotype-specific signal intensities of the genes ($G_iC_j$ effects), which are the effects of interest, gene-specific dye effects ($G_iD_l$ effects) were estimated and t-tested for significance at the 1% level. One hundred and thirty one genes showed a significant $G_iD_l$ effect and were discard from further analysis. For each of the remaining 4,448 genes on the arrays, a t-test on the $G_iC_j$ effects for significant differences ($p<0.05$) was performed. Figure 1 plots the obtained p-values (as the negative log10 of the p-value) against the magnitude of the effect (log2 of estimated fold change). This volcano plot illustrates the substantial difference significance testing can make versus cutoffs made strictly on the basis of the fold change. The two vertical reference lines indicate a 2-fold cutoff for either repression or induction, while the horizontal reference line refers to the p-value cutoff at the 0.05 value. These reference lines divide the plot into six sectors. The 3,535 genes in the lower middle sector have low significance and low fold change, and both methods agree that the corresponding changes are not significant. The 188 genes in the upper left and right sectors have high significance ($p<0.05$) and high fold change ($\geq 2$); 84 of these genes show a significant two-or-more-fold induction of expression, where the remaining 104 genes show a significant two-or-more-fold repression of expression in the E2Fa-DPa plant. Finally, the 715 genes in the upper middle sector represent significant ($p<0.05$) up- or downregulated genes, but with a low ($\leq 2$) fold change. The full dataset of genes can be viewed at http://www.psb.rug.ac.be/E2F, which dataset is incorporated herein by reference.

All the sequences that are 1.3 times upregulated (ratio of more than 1.999) in E2Fa-DPa overexpressing plants are presented in Table 4. All the sequences that are 1.3 times repressed (calculated as 1/ ratio of less than 0.775) are presented in Table 5. Particularly interesting genes that are more than 2-fold upregulated or 2 fold repressed are selected and separately represented in Tables 1 and 2.

***Example 4: sequencing and RT mediated PCR analysis***

The identity of the genes was confirmed by sequencing, and the induction of a random set of genes was confirmed by RT-PCR analysis (Figure 5).

RNA was isolated from plants 8 days after sowing with the Trizol reagent (Amersham Biosciences). First-strand cDNA was prepared from 3 µg of total RNA with the Superscript RT II kit (Invitrogen) and oligo(dT)18 according to the manufacturer's instructions. A 0.25 µl aliquot of the total RT reaction volume (20 µl) was used as a template in a semi-quantitative RT-mediated PCR amplification, ensuring that the amount of amplified product remained in linear proportion to the initial template present in the reaction. From the PCR reaction, 10 µl was separated on a 0.8% agarose gel and transferred onto Hybond N+ membranes (Amersham Biosciences) that were hybridized at 65°C with fluorescein-labeled probes (Gene Images random prime module; Amersham Biosciences). The hybridized bands were detected with the CDP Star detection module (Amersham Biosciences). Primers used were 5'-AAAAAGCAGGCTGTGTCGTACGATCTTCTCCCGG-3' (SEQ ID NO 2757) and 5'-AGAAA GCTGGGTCATGTGATAGGAGAACCAGCG-3' (SEQ ID NO 2758) for E2Fa, 5'-ATAGAA TTCGCTTACATTTTGAAACTGATG-3' (SEQ ID NO 2759) and 5'-ATAGTCGACTCAGCGA GTATCAATGGATCC-3' (SEQ ID NO 2760) for DPa, 5'-CAGATCTTGTTAACCTTGACAT CTCAG-3' (SEQ ID NO 2761) and 5'-GGGTCAAAAGATACAACCACACCAG-3' (SEQ ID NO 2762) for glutamine synthetase (GS), 5'-GGTTTACGAGCTACATGGCCC-3' (SEQ ID NO 2763) and 5'-GAGCAATCCGTTCAGCCTCC-3' (SEQ ID NO 2764) for glutamate synthase (GOGAT), 5'-GCGTTTGACCACTCTTGGAGAC-3' (SEQ ID NO 2765) and 5'-GAACGCCA TTGAGAAAGTCCGC-3' (SEQ ID NO 2766) for histone acetylase HAT B, 5'-GTTACCGG CTCGACTTGAAGATC-3' (SEQ ID NO 2767) and 5'-GAATCGGAGGGAAAGTCTGACG-3' (SEQ ID NO 2768) for LOB domain protein 41, 5'-GTGTGGTTTCCAAGCTTTCCTACG-3' (SEQ ID NO 2769) and 5'-GGTGAAGGGACTAGCCTTGTGG-3' (SEQ ID NO 2770) for isocitrate lyase, 5'-GGGATCAATCCTCAGGAGAAGG-3' (SEQ ID NO 2771) and 5'-CCGTCCATCTTTATTAGCGGCATG-3' (SEQ ID NO 2772) for nitrite reductase (NiR), and 5'-TTACCGAGGCTCCTCTTAACCC-3' (SEQ ID NO 2773) and 5'-ACCACCGATCCAGACA CTGTAC-3' (SEQ ID NO 2774) for actin 2 (ACT2).

***Example 5: Characterization of the genes identified as being involved in E2F/DP regulated cellular processes***

The genes of the present invention identified from the microarray experiment of Example 2 have unique identification numbers (MIPS accession number e.g. At1g57680). The MIPS accession number is widely accepted in this field as it directly refers to the genomic sequence and the location of the sequence in the Arabidopsis thaliana genome. Accession numbers are allocated by the Munich Information Center for Protein Sequences (MIPS) and are stored in the MIPS *Arabidopsis* database. Publicly available sequence and annotation data from all other AGI ("*Arabidopsis* Genome Initiative") groups are included to establish a plant genome database (Schoof H, et al. (2002)). The MIPS *Arabidopsis* database can be accessed via the Internet http://mips.gsf.de/cgi-bin/proj/thal and the database can be searched with the protein entry code (e.g. At1g57680). An example of the type of sequence information and protein domain information that is provided for a certain sequence in the MIPS database is shown Figure 4.

An additional blast search with the genes according to the present invention was performed on public databases containing sequences from other plant species and other organisms. For some of the genes identified by the microarray, significant levels of homology (low E-values) were found with sequences from other organisms (see Tables 1 and 2 with reference to their Genbank accession number). So far, mainly corn and rice homologues were identified, but as more genomes will be sequenced in the future, many more homologues will be identifiable by the person skilled in the art as useful in the methods of the present invention.

***DNA replication and cell cycle genes***

Genes up or downregulated in the *E2Fa-DPa* overexpressing plants can be classified into clear groups according their function (Tables 1 and 2). 14 Genes that are 2-fold or more upregulated belong to the class of genes involved in DNA replication and modification, correlating with the observation that E2Fa-DPa overexpression plants undergo extensive endoreduplication. Most of these genes have previously be shown to be upregulated by *E2F-DP* overexpression in mammalian systems including a putative thymidine kinase, replication factor c, and histone genes (4 different ones). Other E2Fa-DPa induced S phase genes include a linker histone protein, the topoisomerase 6 subunit A and two subunits of the histone acetyltransferase HAT B complex, being HAT B and Msi3. The HAT B complex is responsible for the specific diacethylation of newly synthesized histone H4 during nuclease assembly on newly synthesized DNA (Lusser et al., 1999). Also a DNA methyltransferase responsible for the methylation of cytosine in cells that progress though S phase was identified among upregulated genes.

Besides the overexpressed E2Fa gene (being 90-fold more abundant in the E2FaPa overexpressing plants, compared to control plants), only one cell cycle gene (*CDKB1;1*) shows a 2-fold or more change in expression level upon *E2Fa-DPa* overexpression. CDKB1;1 was previously predicted to be a candidate E2F-DP target by virtue of a consensus E2F-DP-binding site in its promoter (de Jager et al., 2001). Whereas CDKB1;1 activity is maximum at the G2/M transition, its transcript levels start to rise during late S-phase (Porceddu et al., 2001; Menges and Murray, 2002). Upregulation of *CDKB1;1* might therefore be a mechanism to link DNA replication with mitosis.

***Cell wall biogenesis genes***

Four members of the xyloglucan endotransglucosylase (XET) gene family were found to be 2-fold or more upregulated in E2Fa-DPa overexpressing plants, one of them identical to the Meri-5 gene (Medford et al., 1991). XETs are enzymes that modify cell wall components and therefore play a likely role in altering size, shape and physical properties of plant cells. Reversal breakage of the xyloglucan tethers by XETs has been proposed to be a mechanism for allowing cell wall loosening in turgor-driven cell expansion (Campbell and Braam, 1999). However, there are several reasons to believe that E2Fa-DPa induced XETs are not required for cell expansion. First, cells divide more frequently in E2Fa-DPa overexpressing plants, but the overall cell size of the cells is smaller. Therefore, no overall increase in expansion-rates is needed. Second, correlated with the absence of increased cell expansion in the transgenic lines, no induction of genes with a known role in this process, such as expansins, can be seen. Therefore, the hydrolytic activity of the XETs might be required to incorporate the newly synthesized cell walls formed during cytokinesis into the existing cell wall structure. Alternatively, as XET activity has shown to be involved in the postgerminative mobilization of xyloglucan storage reverses in Nasturtium cotelydons (Farkas et al., 1992; Fanutti et al., 1993), induction of XETs in E2Fa-DPa overexpressing plants might relate to polysaccharide breakdown to serve the metabolic and energy needs which are required to synthesize new nucleotides (see below).

Interestingly, two XETs were identified in the set of 2-fold or more downregulated genes. These XETs are more related to each other than to the induced XET proteins. This differential response of XETs towards the E2Fa/DPa induced phenotypes suggests that plant XETs can be classified in at least 2 different functional classes.

***Genes involved in metabolism and biogenesis***

Both the group of up and down regulated genes contains a relative large group of genes involved in metabolism and biogenesis. Most remarkable is the induction of genes involved in nitrogen assimilation, such as nitrate reductase (NIA2) (see Figure 2), glutamine synthetase (GS), and glutamate synthetase (GOGAT). Although not present on the microarray, the nitrite reductase (NiR) gene was found to be induced in the transgenic line, as demonstrated by RT-mediated PCR analysis. Nitrogen and nitrite reductase catalyse the first step in the nitrogen assimilation pathway, whereas glutamine and glutamate synthetase are involved in both the primary assimilation from nitrogen as reassimilation of free ammonium, supplying all plants nitrogen needed for the biosynthesis of amino-acids and other nitrogen-containing compounds.

There are other indications that nitrogen metabolism is altered in E2Fa-DPa overexpressing plants, such as the modification of genes reported to be involved in Medicago induced nodulation (MTN3 and a nodulin-like gene); and the downregulation of genes involved in sulfur assimilation (adenylylsulfate reductase (APR; 2 different genes) and a putative adenine phosphosulfate kinase). Genes involved in sulfur assimilation such as APR have previously been shown to be transcriptionally downregulated during nitrogen deficiency (Koprivova et al., 2000).

Upregulation of nitrogen assimilation genes in E2Fa-DPa overexpressing plants might reflect the need for nitrogen for nucleotide biosynthesis, as purine and pyrimidine bases are nitrogen-rich. If nitrogen assimilation was indeed stimulated by E2Fa/DPa overexpression, two requirements should be fulfilled. Since nitrogen assimilation through the GS/GOGAT pathway requires α-ketogluterate (Lancien et al., 2000), a first requirement is that there should be enough α-ketogluterate to act as acceptor molecule for ammonium. Secondly, because assimilation of nitrogen is energy consuming, the rate of reductant production should be higher in an E2Fa/DPa transgenic than in wild-type plants.

Our micro-array data suggest that in the *E2Fa-DPa* overexpressing plants, α-ketogluterate accumulation is stimulated in different ways. First, α-ketogluterate production is improved by increased photosynthetic activity, as indicated by the 4.7-fold upregulation of large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Figure 2). This results in an accumulation of glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate can be converted into fructuse-1,6-bisphosphate by fructose bisphosphate aldolase. However, a 6-fold downregulation of the fructose bisphosphate aldolase gene rather suggests the conversion of glyceraldehyde-3-phosphate into pyruvate, which can be converted into α-ketogluterate during glycolysis in the citrate cycle. The preferential conversion of glyceraldehyde-3-phosphate into pyruvate in favour of sugars fits the higher need for amino-acids than for sugars for nucleotide

biosynthesis. A shortage for ribose-5-phosphate for nucleotide synthesis is also evident from a downregulation of sucrose-phosphate synthase, resulting in decreased conversion of fructose-6-phosphate and glucose-6-phosphate into sucrose (Figure 2).

A second source of α-ketogluterate is provided in the glyoxylate cycle by the 3.1 fold increase in expression of isocitrate lyase, suggesting an increased lipid turnover in E2Fa-DPa overexpressing plants. Isocistrate lyase activity cleaves isocitrate into glyoxylate and succinate (Figure 2). Whereas the formed glyoxylate can be converted into glycine, which is also required for nucleotide biosynthesis, succinate can be converted into α-ketogluterate in the citrate cycle. A 2.3-fold decrease of the fumarase gene presumably stimulates the conversion of produced α-ketogluterate into glutamate by causing an accumulation of succinate and fumarate, which is also a side product formed during nucleotide biosynthesis (Figure 2).

Assimilation of nitrogen is energy consuming. When rates of nitrate reduction are high, this pathway becomes the major sink for reductant. About 10% of the electron flux in photosynthesizing leaves is used for nitrate reduction. The amount of required reductant, which in leaves originates from electronic photosynthetic electron transport, is therefore expected to be higher in the E2Fa-DPa transgenics. Correspondingly, several components of the chloroplast electron transport chain and associated ATP-synthesing apparatus, such as cytochrome B6, a PSII subunit and the ATPase epsilon subunit are upregulated in the transgenic plants. Increased expression of the protochlorophyllide reductase precursor suggests that an increase in chlorophyll biosynthesis is stimulated in E2Fa-DPa overexpressing plants.

Famine of nitrogen has a putative impact on amino-acid biosynthesis, as three different amino-acid aminotransferases, are downregulated in E2Fa-DPa overexpressing plants. Accompanied with a putative decreased aminotransferase activity is the observed reduction in expression of an enzyme involved in pyridoxine biosynthesis. Shortage of nitrogen-rich amino-acids is also evident from reduced expression of genes encoding vegetative storage proteins (VSP1and VSP2); and ERD10, a protein with a compositional bias towards glu (Kiyosue et al., 1994). Additional evidence for amino acid shortage comes from downregulation of a myrosinase-binding protein and cytochrome P450 monooxygenase CYP83A1. Both proteins are involved in the biosynthesis of glucosinolates, being nitrogen and sulfur containing products derived from amino-acids (Wittstock and Halkier, 2002).

***Transcription factors and signal transduction***

A total of 4 transcription factors were identified among the genes being 2-fold or more upregulated, including two homeobox domain transcription factors. Among them the anthocyaninless2 (ANL2) gene was identified, which is involved in anthocyanin accumulation in subepidermal leaf cells (Kubo et al., 1999). The lack of an obvious increase in anthocyanin accumulation in E2Fa-DPa overexpressing plants suggests a role for the ANL2 protein in plant development different from anthocyanin production. This hypothesis is substantiated by the observation that anl2 mutant plants contain extra cells in the root between the cortical and epidermal layers (Kubo et al., 1999).

The second upregulated homeobox domain transcription factor is Atbh-6. Expression of Atbh-6 is restricted to regions of cell division and/or differentiation and has been shown to be inducible by water stress and ABA (Soderman et al., 1999). Other putative ABA sensitive genes can be recognized among the E2Fa-DPa induced clones, as well as the cold regulated protein COR6.6, a seed imbitition-like protein and a dormancy-associated protein. Here again, changes in expression level of these genes might be correlated with modifications in carbon metabolism. A link between ABA and sugar signaling is evident from the identification of several loci involved in both sugar and hormonal responses (Finkelstein and Gibson, 2002). Alternatively, it might be the occurrence of enhanced endoreduplication and/or cell division itself that causes a change in the osmotic potential.

Among the downregulated transcription factors a DOF family member is present. Many DOF transcription factors are participating in the regulation of storage protein genes and genes involved in carbon metabolism (Gualberti et al., 2002). Its downregulation might therefore be linked with the shortage of amino-acids due to the high demand of nitrogen for nucleotide biosynthesis.

Other regulatory genes modified in E2Fa-DPa overexpressing plants include protein kinases, several putative receptor kinases, a putative phytochrome A, and WD-40 repeat containing proteins (Tables 1 and 2). Interestingly, a SNF1-like kinase is downregulated 2-fold in E2Fa-DPa overexpressing plants. In addition to its proposed role in sugar signaling, the SNF1 kinase also negatively regulates the activity of plant nitrate reductase (Smeekens, 2000).

***Example 6: Endoreduplication levels of E2Fa-DPa plants are nitrogen dependent***

The modified expression of a large number of metabolic and regulatory genes, directly or indirectly linked to nitrogen metabolism, suggests a direct relationship between the high endoreduplication levels found in the E2Fa/DPa transgenic plants and nitrogen availability. To test this hypothesis, wild type and transgenic plants were grown on medium containing

different levels of ammoniumnitrate, ranging from 0.1 to 50 mM. Eight days after germination ploidy levels in these plants was determined by flow cytometry. Increasing ammoniumnitrate levels hardly had an effect on the ploidy distribution pattern in wild type plants (Figure 3A). In contrast, in the E2Fa-DPa transgenic plants increasing ammoniumnitrate levels resulted in a reproducible and significant increase in the amount of 32C and 64C nuclei (Figure 3B). Comparing the lowest with the highest concentration of ammonimumnitrate, an increase of 32C from 2.0 (± 0.3) % to 6.5 (± 1.5) %, and of 64C from 0.7 (±0.3) % to 2 (± 0.5) % can be seen. Increasing ammonium levels did not have any effect on the plant phenotype, as plants remained small with curled leaves on all concentrations of nitrogen tested. These data indicate that the endoreduplication levels in the E2Fa-DPa overexpressing plants are limited by nitrogen availability, and that an excess of nitrogen is incorporated into new DNA than in other nitrogen containing compounds.

***Example 7: Promoter analysis of E2Fa-DPa regulated genes***

***Promoter analysis***

The intergenic sequence corresponding to the promoter area of each gene spotted on the microarray was extracted from genomic sequences. These genomic sequences are easily accessible for example from the MIPS MatDB database (http://mips.gsf.de/proj/thal/db/). From those intergenic sequences, up to 500bp upstream of the ATG start codon were extracted and subjected to motif searches in order to retrieve potential E2F elements. Both position and frequency of occurrence was determined using the publicly available execuTable of MatInspector (version 2.2) using matrices extracted from PlantCARE and matrices made especially for this particular analysis (Lescot et al., 2002). The relevance of each motif was evaluated against a background consisting of all the sequences from the dataset.

***Results***

To distinguish in the present data set the putative direct target genes of E2Fa-DPa from the secondary induced genes, the first 500 bp upstream of the ATG start codon of the genes with 2-fold or more change in expression was scanned for the presence of a E2F-like binding site matching the sequence (A/T)TT(G/C)(G/C)C(G/C)(G/C) (SEQ ID NO 2775). Of all the different permutations possible, only the TTTCCCGC (SEQ ID NO 2776) element was statistically enriched in the set of E2Fa-DPa upregulated genes, suggesting it is the preferred binding site of the E2Fa-DPa complex (Table 3). Moreover, target genes containing this element belong mainly to the group of genes involved in DNA replication and modification, being the main group of target genes in mammalian systems. These data illustrate that the TTTCCCGC sequence is the most likely cis element recognized by E2Fa-DPa. The observation that not all genes having this DNA sequence in their promoter suggests that the presence of the

TTTCCCCGC motif is not sufficient to make a gene responsive towards E2Fa-DPa, and that E2Fa-DPa co-operates with other factors to activate transcription.

It is not excluded that genes without an E2F-like-binding site are not directly activated by E2Fa-DPa. Chromatin immunoprecipitation experiments have shown that mammalian E2F factors can bind to promoters without a clear E2F recognition motif (Kiyosue *et al.*, 1994), suggesting that E2FDP might recognize non-canonical binding sites, or might be recruited by promoters through the association of other factors. In this respect, the *Chlorella vulgaris* nitrate reductase gene, of which the *Arabidopsis* homologue was shown herein to be induced by E2F-DPa, binds an E2F-DP complex, although a clear consensus binding site is lacking (Cannons and Shiflett, 2001).

***E2F can activate as well as repress promoter activity.***

In the Nicotiana benthamiana PCNA promoter a E2F sequence was identified acting as a negative regulatory element during development (Egelkrout et al., 2001). Also the tobacco ribonucleotide reductase small subunit gene contains a E2F element working as a repressor outside the S-phase (Chaboute et al., 2000). In the set of downregulated genes no particular enrichment of a specific E2F sequence could be seen (Table 3). Therefore the inventors believe that the E2Fa-DPa complex mainly works as a transcriptional activator, and that other E2F-DP complexes are involved in E2F-mediated transcriptional repression.

***Example 8: individual characterization of some genes identified by the method of the present invention***

The genes characterized hereunder, are particularly useful for making plants with improved growth characteristics. These preferred genes are introduced into a plant and upregulated or downregulated in order to simulate E2Fa/DPa effects and/or to alter one or more characteristics of a plant. The particular growth characteristic that may be influenced by these genes, is described in the following paragraphs by reference to the biological function of that particular gene.

***At1g07000 showing homology to leucine zipper***

At1g07000 is a potential leucine zipper that is not preceded by a basic domain. The leucine zipper consists of repeated leucine residues at every seventh position and mediates protein dimerization as a prerequisite for DNA-binding. The leucines are directed towards one side of an alpha-helix. The leucine side chains of two polypeptides are thought to interdigitate upon dimerization (knobs-into-holes model). The leucine zipper may dictate dimerization specificity.

Leucine zippers are DNA binding protein with dimerization properties, having important functions in development and stress tolerance in plants.

***At1g09070 showing homology to Soybean Cold Regulate gene SRC2***

This genes and its expressed protein is predicted in *Arabidopsis*, rice, corn, soybean, however, based on a homology search using the BLAST program, no functional homologue was known, not even a clear animal homologue, so no clear function can be predicted for this gene or protein (Takahashi,R. and Shimosaka,E. (1997)).

***At1g21690 showing homology to Replication factor***

Replication factor C (RFC) is a pentameric complex of five distinct subunits that functions as a clamp loader, facilitating the loading of proliferating cell nuclear antigen (PCNA) onto DNA during replication and repair. More recently the large subunit of RFC, RFC (p140), has been found to interact with the retinoblastoma (Rb) tumor suppressor and the CCAAT/enhancer-binding protein alpha (C/EBPalpha) transcription factor. It is reported that RFC (p140) associates with histone deacetylase activity and interacts with histone deacetylase 1 (HDAC1) (Anderson, L. A. and Perkins, N. D. (2002); Furukawa,T. et al. (2001)) RFC is poorly known in plants. It can be important for development for modulating gene expression during cell cycle at S phase, or through chromatin regulation.

***At1g23030 showing homology to Armadillo protein***

Members of the armadillo (arm) repeat family of proteins are implicated in tumorigenesis, embryonic development, and maintenance of tissue integrity. ARM proteins participate in linking cytoskeleton to membrane proteins and structures. These proteins share a central domain that is composed of a series of imperfect 45 amino acid repeats. Armadillo family members reveal diverse cellular locations reflecting their diverse functions. A single protein exerts several functions through interactions of its armadillo repeat domain with diverse binding partners. The proteins combine structural roles as cell-contact and cytoskeleton-associated proteins and signaling functions by generating and transducing signals affecting gene expression. The study of armadillo family members has made it increasingly clear that a distinction between structural proteins on the one hand and signaling molecules on the other is rather artificial. Instead armadillo family members exert both functions by interacting with a number of distinct cellular-binding partners. Proteins of the armadillo family are involved in diverse cellular processes in higher eukaryotes. Some of them, like armadillo, beta-catenin and plakoglobins have dual functions in intercellular junctions and signalling cascades. Others belonging to the importin-alpha-subfamily are involved in NLS (Nuclear localization signal) recognition and nuclear transport, while some members of the armadillo family have as yet

unknown functions. (Wang, Y. X. et al. (2001); Hatzfeld, M. (1999). ARM proteins are key protein binding units that are involved at several steps during development. Some are specific to the cell cycle APC degradation complex. These type of genes have been poorly studied in plants, some have been involved in light and gibberellin signaling in potato.

***At1g27500 showing homology to Kinesin light chain.***

The motor protein kinesin is a heterotetramer composed of two heavy chains of approximately 120 kDa and two light chains of approximately 65 kDa protein. Kinesin motor activity is dependent on the presence of ATP and microtubules. Conventional kinesin is prevented from binding to microtubules (MTs) when not transporting cargo. The function of LC kinesin is to keep kinesin in an inactive ground state by inducing an interaction between the tail and motor domains of HC; activation for cargo transport may be triggered by a small conformational change that releases the inhibition of the motor domain for MT binding. This protein is important to regulate movement controlled by microtubules within the cytoplasm, for example the flux of vesicles between the different cell membrane compartments.

***At1g72180 showing homology to Putative receptor protein kinase***

Plant receptor-like kinases (RLKs) are transmembrane proteins with putative amino-terminal extracellular domains and carboxyl-terminal intracellular kinase domains, with striking resemblance in domain organization to the animal receptor tyrosine kinases such as epidermal growth factor receptor. The recently sequenced *Arabidopsis* genome contains more than 600 RLK homologs. Although only a handful of these genes have known functions and fewer still have identified ligands or downstream targets, the studies of several RLKs such as CLAVATA1, Brassinosteroid Insensitive 1, Flagellin Insensitive 2, and S-locus receptor kinase provide much-needed information on the functions mediated by members of this large gene family. RLKs control a wide range of processes, including development, disease resistance, hormone perception, and self-incompatibility. Combined with the expression studies and biochemical analysis of other RLKs, more details of RLK function and signaling are emerging.

**At1g72900 showing homology to Disease resistance protein (TIR virus resistance protein)**

The TIR gene has been described by Kroczynska, B. et al. (1999).

***At2g30590 showing homology to WRKY transcription factor (TolI/interleukin-1 receptor-like protein)***

The sequence shows homology to tomato Cf-9 resistance gene Avr9/Cf-9 rapidly elicited protein 4 (NL27) (Hehl, R. et al. (1998)). WRKY proteins are a large group of transcription factors restricted to the plant kingdom. WRKY proteins are a recently identified class of DNA-binding proteins that recognize the TTGAC(C/T) W-box elements found in the promoters of a large number of plant defense-related genes (Dong and Chen, 2003). It has been found that the majority are responsive both to pathogen infection and to salicylic acid. The functions of all other WRKY genes revealed to date involve responses to pathogen attack, mechanical stress, and senescence (Dong and Chen, 2003).

***At1g80530 showing homology to Nodulin***

Infection of soybean roots by nitrogen-fixing Bradyrhizobium japonicum leads to expression of plant nodule-specific genes known as nodulins. Nodulin 26, a member of the major intrinsic protein/aquaporin (AQP) channel family, is a major component of the soybean symbiosome membrane (SM) that encloses the rhizobium bacteroid. These results indicate that nodulin 26 is a multifunctional AQP that confers water and glycerol transport to the SM, and likely plays a role in osmoregulation during legume/rhizobia symbioses (Dean et al. (1999). Rice (*Oryza sativa var. Nipponbare*) possesses two different homologues of the soybean early nodulin gene GmENOD93 (GmN93), OsENOD93a (homology of 58.2% to GmENOD93), OsENOD93b (homology of 42.3%). In intact rice tissues, OsENOD93b was most abundantly expressed in roots and at much lower levels in etiolated and green leaves, whereas the expression of OsENOD93a was very low in roots and etiolated leaves, and was not detected in green leaves. The level of OsENOD93a expression was enhanced markedly in suspension-cultured cells, whereas that of OsENOD93b did not increase (Reddy et al. (1998)). Homologues of genes that are produced in response to infection of soybean roots by bacteria are also present in other plants such rice. Their function is largely unknown, some functional homologues are identified such as a water channel involved in osmoregulation.

***At2g34770 showing homology to Fatty acid hydroxylase***

This gene has been described in Matsuda et al. (2001). A common feature of the membrane lipids of higher plants is a large content of polyunsaturated fatty acids, which typically consist of dienoic and trienoic fatty acids. Two types of omega-3 fatty acid desaturase, which are present in the plastids and in the endoplasmic reticulum (ER), respectively, are responsible for the conversion of dienoic to trienoic fatty acids. To establish a system for investigating the tissue-specific, and hormone-regulated expression of the ER-type desaturase gene (FAD3), transgenic plants of *Arabidopsis thaliana* (L.) Heynh. containing the firefly luciferase gene

(LUC) fused to the FAD3 promoter (FAD3::LUC) were constructed. The results as discussed in this report suggest that the expression of ER-type desaturase is regulated through synergistic and antagonistic hormonal interactions, and that such hormonal regulation and the tissue specificity of the expression of this gene are further modified in accordance with the growth phase in plant development (Wellesen K, et al. (2001); Kachroo P, et al. (2001); Kahn, R. A. et al. (2001); Smith, M. et al. (2000)).

***At2g43402 showing homology to Cinnamoyl CoA reductase***

CCR enzyme is involved in lignification. The CCR transcript is expressed in lignified organs, i.e. root and stem tissues, and is localized mainly in young differentiating xylem. Also, monolignols may be precursors of end products other than lignins. CCR catalyses the conversion of cinnamoyl-CoAs into their corresponding cinnamaldehydes, i.e. the first step of the phenylpropanoid pathway specifically dedicated to the monolignol biosynthetic branch. The two genes are differentially expressed during development and in response to infection. AtCCR1 is preferentially expressed in tissues undergoing lignification. In contrast, AtCCR2, which is poorly expressed during development, is strongly and transiently induced during the incompatible interaction with Xanthomonas campestris pv. Campestris leading to a hypersensitive response. Altogether, these data suggest that AtCCR1 is involved in constitutive lignification whereas AtCCR2 is involved in the biosynthesis of phenolics whose accumulation may lead to resistance (Lauvergeat et al. (2001)). This protein is involved during development, increase in growth diameter, lignification of vascular strands and interfascicular fibers.

***At2g47440 showing homology to Tetratricopeptide repeat protein***

The tetratricopeptide repeat (TPR) is found in many proteins performing a wide variety of functions, the TPR domain itself is believed to be a general protein recognition module. Different proteins may contain from 3 to 16 tandem TPR motifs (34 amino acid sequence). It has been shown that some proteins contain a TPR repeat are cell cycle regulated.

***At3g23750 showing homology to Receptor like kinase TMK***

The kinase domain of NtTMK1 contained all of 12 subdomains and invariant amino acid residues found in eukaryotic protein kinases. The extracellular domain contained 11 leucine-rich repeats, which have been implicated in protein-protein interactions. The amino acid sequence of NtTMK1 exhibited high homology with those of TMK1 of *Arabidopsis* and TMK of rice in both kinase and extracellular domains, suggesting that NtTMK1 is a TMK homologue of tobacco. The NtTMK1 transcripts were present in all major plant organs, but its level varied in different developmental stages in anthers and floral organs. NtTMK1 mRNA accumulation in

leaves was stimulated by CaCl2, methyl jasmonate, wounding, fungal elicitors, chitins, and chitosan. The NtTMK1 mRNA level also increased upon infection with tobacco mosaic virus (Cho and Pai (2000)). This protein is involved in different aspects of development and disease resistance.

***At3g61460 showing homology to RING H2***

RING-finger proteins contain cysteine-rich, zinc-binding domains and are involved in the formation of macromolecular scaffolds important for transcriptional repression and ubiquitination. RING H2 act as E3 ubiquitin-protein ligases and play critical roles in targeting the destruction of proteins of diverse functions in all eukaryotes, ranging from yeast to mammals. The *Arabidopsis* genome contains a large number of genes encoding RING finger proteins. A small group is constituted by more than 40 RING-H2 finger proteins that are of small size, not more than 200 amino acids, and contain no other recognizable protein-protein interaction domain(s). This type of genes is very important for several aspect of development, regulation of developmental proteins, cell cycle proteins.

***At4g00730 showing homology to Homeodomain AHDP (antocyaninless 2 )***

This is a homeodomain transcription factor; similar to ATML1 and is very conserved and has epidermis specific expression. This sequence shows also homology to *Zea mays* mRNA for OCL3 protein (Ingram, G. C. et al. (2000)).

***At4g13940 showing homology to adenosylhomocysteinase (Glutathione dependent formaldehyde dehydrogenase)***

Glutathione-dependent formaldehyde dehydrogenase was described in Sakamoto, A. et al. (2002), *Arabidopsis* glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase. S-Nitrosoglutathione (GSNO), an adduct of nitric oxide (NO) with glutathione, is known as a biological NO reservoir. Heterologous expression in *Escherichia coli* of a cDNA encoding a glutathione-dependent formaldehyde dehydrogenase of *Arabidopsis* thaliana showed that the recombinant protein reduces GSNO. The identity of the cDNA was further confirmed by functional complementation of the hypersensitivity to GSNO of a yeast mutant with impaired GSNO metabolism. This is the first demonstration of a plant GSNO reductase, suggesting that plants possess the enzymatic pathway that modulates the bioactivity and toxicity of NO.

***At4g35050 showing homology to WD40 MSI3***

Members of the MSI/RbAp sub-family of WD-repeat proteins are widespread in eukaryotic organisms and form part of multiprotein complexes that are involved in various biological

pathways, including chromatin assembly, regulation of gene transcription, and cell division. The *Zea mays* RbAp-like protein (ZmRbAp1) binds acetylated histones H3 and H4 and suppresses mutations that have a negative effect on the Ras/cAMP pathway in yeast. The ZmRbAp genes form a gene family and are expressed in different tissues of *Z. mays L.* plants. Determination of its expression pattern during maize seed development revealed that ZmRbAp transcripts are abundant during the initial stages of endosperm formation. In addition, the transcripts are specifically localized in shoot apical meristem and leaf primordia of the embryo. ZmRbAp genes play a role in early endosperm differentiation and plant development (Rossi et al. (2001)). Also Rb proteins are known to be involved in multi-protein complexes; there are Rb binding protein characterized; and Rb plays a role in chromatin remodeling and cell cycle control and is important in development and growth of organs. The retinoblastoma (RB) protein regulates G1 progression and functions through its association with various cellular proteins. Two closely related mammalian RB binding proteins, RbAp48 and RbAp46, share sequence homology with the Msi1 protein of yeast. MSI1 is a multicopy suppressor of a mutation in the IRA1 gene involved in the Ras-cAMP pathway that regulates cellular growth. Human RbAp48 is present in protein complexes involved in histone acetylation and chromatin assembly. Four plant RbAp48- and Msi1-like proteins have been identified: one from tomato, LeMSI1, and three from *Arabidopsis*. LeMSI1 can function as a multicopy suppressor of the yeast ira1 mutant phenotype. The LeMSI1 protein localizes to the nucleus and binds to a 65-kD protein in wild-type as well as ripening inhibitor (rin) and Neverripe (Nr) tomato fruit. LeMSI1 also binds to the human RB protein and the RB-like RRB1 protein from maize, indicating that this interaction is conserved between plants and animals (Ach et al. (1997)).

***At4g36670 showing homology to Sugar transporter***

The ERD6 clone is expressed after exposure to dehydration stress for 1 h. The ERD6 is related to sugar transporters of bacteria, yeasts, plants and mammals. Hydropathy analysis revealed that ERD6 protein has 12 putative transmembrane domains and a central hydrophilic region. Sequences that are conserved at the ends of the 6th and 12th membrane-spanning domains of sugar transporters are also present in ERD6. ERD6 gene is a member of a multigene family in the *Arabidopsis* genome. The expression of the ERD6 gene was induced not only by dehydration but also by cold treatment (Kiyosue et al. (1998)) .

***At5g01870 showing homology to Lipid transfer protein***

Nonspecific lipid transfer proteins (LTPs) from plants are characterized by their ability to stimulate phospholipid transfer between membranes *in vitro*. However, because these proteins are generally located outside of the plasma membrane, it is unlikely that they have a similar role *in vivo*. The LTP1 promoter was active early in development in protoderm cells of

embryos, vascular tissues, lignified tips of cotyledons, shoot meristem, and stipules. In adult plants, the gene was expressed in epidermal cells of young leaves and the stem. In flowers, expression was observed in the epidermis of all developing influorescence and flower organ primordia, the epidermis of the siliques and the outer ovule wall, the stigma, petal tips, and floral nectaries of mature flowers, and the petal/sepal abscission zone of mature siliques. Consistent with a role for the LTP1 gene product in some aspect of secretion or deposition of lipophilic substances in the cell walls of expanding epidermal cells and certain secretory tissues. The LTP1 promoter region contained sequences homologous to putative regulatory elements of genes in the phenylpropanoid biosynthetic pathway, suggesting that the expression of the LTP1 gene may be regulated by the same or similar mechanisms as genes in the phenylpropanoid pathway (Thoma, S. et al. (1994)). More background knowledge to this type of genes can be found in the following references: Clark, A. M. et al., (1999); Toonen, M. A. et al. (1997); Molina, A. (1997); Thoma, S. et al. (1994).

***At5g02820 showing homology to SPO like***

Plant steroid hormones, such as brassinosteroids (BRs), play important roles throughout plant growth and development. Plants defective in BR biosynthesis or perception display cell elongation defects and severe dwarfism. Two dwarf mutants named bin3 and bin5 with identical phenotypes to each other display some characteristics of BR mutants and are partially insensitive to exogenously applied BRs. In the dark, bin3 or bin5 seedlings are de-etiolated with short hypocotyls and open cotyledons. Light-grown mutant plants are dwarfs with short petioles, epinastic leaves, short inflorescence stems, and reduced apical dominance. BIN3 and BIN5 were cloned and show that BIN5 is one of three putative *Arabidopsis* SPO11 homologs (AtSPO11-3) that also shares significant homology to archaebacterial topoisomerase VI (TOP6) subunit A, whereas BIN3 represents a putative eukaryotic homolog of TOP6B. The pleiotropic dwarf phenotypes of bin5 establish that, unlike all of the other SPO11 homologs that are involved in meiosis, BIN5/AtSPO11-3 plays a major role during somatic development. Furthermore, microarray analysis of the expression of about 5500 genes in bin3 or bin5 mutants indicates that about 321 genes are down-regulated in both of the mutants, including 18 of 30 BR-induced genes. These results suggest that BIN3 and BIN5 may constitute an *Arabidopsis* topoisomerase VI that modulates expression of many genes, including those regulated by BRs (Yin Y et al. (2002)). More background information on this type of gene can be found in the following references: Soustelle, C. et al. (2002); Kee, K. and Keeney, S. (2002); Hartung, F. and Puchta, H. (2001); Grelon, M. et al. (2001).

***At5g14420 showing homology to copine I (phospholipid binding protein)***

The copines are a newly identified class of calcium-dependent, phospholipid binding proteins that are present in a wide range of organisms, including *Paramecium*, plants, *Caenorhabditis elegans*, mouse, and human. However, the biological functions of the copines are unknown. A humidity-sensitive copine mutant was made in *Arabidopsis* and under non-permissive, low-humidity conditions, the cpn1-1 mutant displayed aberrant regulation of cell death that included a lesion mimic phenotype and an accelerated hypersensitive response (HR). However, the HR in cpn1-1 showed no increase in sensitivity to low pathogen titers. Low-humidity-grown cpn1-1 mutants also exhibited morphological abnormalities, increased resistance to virulent strains of *Pseudomonas* syringae and *Peronospora parasitica*, and constitutive expression of pathogenesis-related (PR) genes. Growth of cpn1-1 under permissive, high-humidity conditions abolished the increased disease resistance, lesion mimic, and morphological mutant phenotypes but only partially alleviated the accelerated HR and constitutive PR gene expression phenotypes. The disease resistance phenotype of cpn1-1 suggests that the CPN1 gene regulates defense responses. Alternatively, the primary function of CPN1 may be the regulation of plant responses to low humidity, and the effect of the cpn1-1 mutation on disease resistance may be indirect (Jambunathan et al. (2001)). *Arabidopsis* growth over a wide range of temperatures requires the BONZAI1 (BON1) gene because bon1 null mutants make miniature fertile plants at 22°C but have wild-type appearance at 28°C. The expression of BON1 and a BON1-associated protein (BAP1) is modulated by temperature. Thus BON1 and BAP1 may have a direct role in regulating cell expansion and cell division at lower temperatures. BON1 contains a Ca(2+)-dependent phospholipid-binding domain and is associated with the plasma membrane. It belongs to the copine gene family, which is conserved from protozoa to humans. The data here obtained suggest that this gene family may function in the pathway of membrane trafficking in response to external conditions (Hua et al. (2001)). The major calcium-dependent, phospholipid-binding protein obtained from extracts of *Paramecium tetraurelia*, named copine, had a mass of 55 kDa, bound phosphatidylserine but not phosphatidylcholine at micromolar levels of calcium but not magnesium, and promoted lipid vesicle aggregation. Current sequence databases indicate the presence of multiple copine homologs in green plants, nematodes, and humans. The full-length sequences reveal that copines consist of two C2 domains at the N terminus followed by a domain similar to the A domain that mediates interactions between integrins and extracellular ligands. The association with secretory vesicles, as well the general ability of copines to bind phospholipid bilayers in a calcium-dependent manner, suggests that these proteins may function in membrane trafficking (Creutz et al. (1998)).

***At5g49160 showing homology to cytosine methyltransferase***

DNMT3L is a regulator of imprint establishment of normally methylated maternal genomic sequences. DNMT3L shows high similarity to the de novo DNA methyltransferases, DNMT3A and DNMT3B, however, the amino acid residues needed for DNA cytosine methyltransferase activity have been lost from the DNMT3L protein sequence. Apart from methyltransferase activity, Dnmt3a and Dnmt3b serve as transcriptional repressors associating with histone deacetylase (HDAC) activity. DNMT3L can also repress transcription by binding directly to HDAC1 protein. PHD-like zinc finger of the ATRX domain is the main repression motif of DNMT3L, through which DNMT3L recruits the HDAC activity needed for transcriptional silencing. DNMT3L as a co-repressor protein and suggest that a transcriptionally repressed chromatin organisation through HDAC activity is needed for establishment of genomic imprints (Aapola et al. (2002)). More background information on this type of gene can be found in Chen, T. et al. (2002); Bartee, L. and Bender, J. (2001); Freitag M. et al. (2002). In *Arabidopsis* a SWI2/SNF2 chromatin remodeling factor-related protein DDM1 and a cytosine methyltransferase MET1 is required for maintenance of genomic cytosine methylation. Mutations in either gene cause global demethylation. There are also effects of these mutations on the PAI tryptophan biosynthetic gene family, which consists of four densely methylated genes arranged as a tail-to-tail inverted repeat plus two unlinked singlet genes. The methylation mutations caused only partial demethylation of the PAI loci: ddm1 had a strong effect on the singlet genes but a weaker effect on the inverted repeat, whereas met1 had a stronger effect on the inverted repeat than on the singlet genes. The double ddm1 met1 mutant also displayed partial demethylation of the PAI genes, with a pattern similar to the ddm1 single mutant. To determine the relationship between partial methylation and expression for the singlet PAI2 gene a novel reporter strain of *Arabidopsis* was constructed, in which PAI2 silencing could be monitored by a blue fluorescent plant phenotype diagnostic of tryptophan pathway defects. This reporter strain revealed that intermediate levels of methylation correlate with intermediate suppression of the fluorescent phenotype. Other background information can be found in Finnegan, E. J. and Kovac K. A. (2000). Plant DNA methyltransferases. DNA methylation is an important modification of DNA that plays a role in genome management and in regulating gene expression during development. Methylation is carried out by DNA methyltransferases which catalyse the transfer of a methyl group to bases within the DNA helix. Plants have at least three classes of cytosine methyltransferase which differ in protein structure and function. The METI family, homologues of the mouse Dnmtl methyltransferase, most likely function as maintenance methyltransferases, but may also play a role in *de novo* methylation. The chromomethylases, which are unique to plants, may preferentially methylate DNA in heterochromatin; the remaining class, with similarity to Dnmt3 methyltransferases of mammals, are putative *de novo* methyltransferases. The various classes of methyltransferase

may show differential activity on cytosines in different sequence contexts. Chromomethylases may preferentially methylate cytosines in CpNpG sequences while the *Arabidopsis* METI methyltransferase shows a preference for cytosines in CpG sequences. Additional proteins, for example DDM1, a member of the SNF2/SWI2 family of chromatin remodeling proteins, are also required for methylation of plant DNA.

***At5g54940 showing homology to Translation initiation factor (translational initiation factor eIF1),***

Protein synthesis has not been considered to be fundamental in the control of cell proliferation. However, data are emerging on the involvement of this process in cell growth and tumorigenesis. Protein biosynthesis is a central process in all living cells. It is one of the last steps in the transmission of genetic information stored in DNA on the basis of which proteins are produced to maintain the specific biological function of a given cell. Protein synthesis takes place on ribosomal particles where the genetic information transcribed into mRNA is translated into protein. The process of protein synthesis on the ribosome consists of three phases: initiation, elongation and termination. Brassinosteroids (BRs) regulate the expression of numerous genes associated with plant development, and require the activity of a Ser/Thr receptor kinase to realize their effects. In animals, the transforming growth factor-beta (TGF-beta) family of peptides acts via Ser/Thr receptor kinases to have a major impact on several pathways involved in animal development and adult homeostasis. TGF-beta receptor-interacting protein (TRIP-1) was previously shown by others to be an intracellular substrate of the TGF-beta type II receptor kinase which plays an important role in TGF-beta signaling. TRIP-1 is a WD-repeat protein that also has a dual role as an essential subunit of the eukaryotic translation initiation factor eIF3 in animals, yeast and plants, thereby revealing a putative link between a developmental signaling pathway and the control of protein translation. In yeast, expression of a TRIP-1 homolog has also been closely associated with cell proliferation and progression through the cell cycle. Transcript levels of TRIP-1 homologs in plants are regulated by BR treatment under a variety of conditions, and transgenic plants expressing antisense TRIP-1 RNA exhibit a broad range of developmental defects, including some that resemble the phenotype of BR-deficient and -insensitive mutants. This correlative evidence suggests that a WD-domain protein with reported dual functions in vertebrates and fungi might mediate some of the molecular mechanisms underlying the regulation of plant growth and development by BRs (Jiang and Clouse (2001)). The *Arabidopsis* COP9 signalosome is a multisubunit repressor of photomorphogenesis that is conserved among eukaryotes. This complex may have a general role in development. association between components of the COP9 signalosome (CSN1, CSN7, and CSN8) and two subunits of eukaryotic translation initiation factor 3 (eIF3), eIF3e (p48, known also as INT-6) and eIF3c

(p105). AteIF3e coimmunoprecipitated with CSN7, and eIF3c coimmunoprecipitated with eIF3e, eIF3b, CSN8, and CSN1. eIF3e directly interacted with CSN7 and eIF3c. eIF3e and eIF3c are probably components of multiple complexes and that eIF3e and eIF3c associate with subunits of the COP9 signalosome, even though they are not components of the COP9 signalosome core complex. This interaction may allow for translational control by the COP9 signalosome (Yahalom et al. (2001)).

***At5g56740 showing homology to Histone acetyl transferase HATB***

Transforming viral proteins such as E1A which force quiescent cells into S phase have two essential cellular target proteins, Rb and CBP/p300. Rb regulates the G1/S transition by controlling the transcription factor E2F. CBP/p300 is a transcriptional co-activator with intrinsic histone acetyl-transferase activity. This activity is regulated in a cell cycle dependent manner and shows a peak at the G1/S transition. CBP/p300 is essential for the activity of E2F, a transcription factor that controls the G1/S transition. It was found that CBP HAT activity is required both for the G1/S transition and for E2F activity. Thus CBP/p300 seems to be a versatile protein involved in opposing cellular processes, which raises the question of how its multiple activities are regulated (Ait-Si-Ali, S. et al (2000)). The BRCA2 is a histone acetyltransferase. Two potential functions of BRCA2 were proposed which includes role in the regulation of transcription and also in DNA repair. Forty-five-amino acid region encoded by exon 3 of BRCA2 was shown to have transcriptional activation function. Recent studies of the several enzymes involved in acetylation and deacetylation of histone residues have revealed a possible relationship between gene transcriptional activation and histone acetylation. Since BRCA2 appear to function as a transcriptional factor, Histone acetyl transferase (HAT) activity of BRCA2 was tested. Also, evidence that BRCA2 has intrinsic HAT activity, which maps to the amino-terminal region of BRCA2, was presented. It was demonstrated that BRCA2 proteins acetylate primarily H3 and H4 of free histones. These observations suggest that HAT activity of BRCA2 may play an important role in the regulation of transcription and tumor suppressor function (Siddique et al. (1998)). These types of genes are very important for regulation of genes involved in development, cell cycle control, and chromatin structure.

***At5g61520 showing homology to STP3 sucrose transporter***

For developing seeds of grain legumes, photoassimilates released to the seed apoplasm from maternal seed coats are retrieved by abaxial epidermal and subepidermal cells (dermal cell complexes) of cotyledons followed by symplasmic passage to their underlying storage parenchyma cells. In some species, the cells of these complexes differentiate into transfer cells (e.g. broad bean and pea, Patrick and Offler,2001). Sucrose is a major component of the photoassimilates delivered to cotyledons (Patrick and Offler, 2001; Weber et al., 1997b).

Sucrose transporter (SUT) genes have been cloned, and functionally characterized as sucrose/H+ symporters, from developing cotyledons of broad bean (VfSUT1, Weber et al., 1997a) and pea (PsSUT1, Tegeder et al., 1999). SUTs and P-type H+-ATPases have been shown to co-localize to plasma membranes of dermal cell complexes in developing cotyledons of broad bean (Harrington et al., 1997; Weber et al., 1997a) and French bean (Tegeder et al., 2000). In contrast, for pea cotyledons, SUT is also present in storage parenchyma cells, but is 4-fold less active than SUT(s) localized to epidermal transfer cells (Tegeder et al., 1999). These type of genes are Important for seed filling.

***At5g66210 showing homology to Calcium dependent protein kinase***

In plants, numerous Ca(2+)-stimulated protein kinase activities occur through calcium-dependent protein kinases (CDPKs). These novel calcium sensors are likely to be crucial mediators of responses to diverse endogenous and environmental cues. However, the precise biological function(s) of most CDPKs remains elusive. The *Arabidopsis* genome is predicted to encode 34 different CDPKs. The *Arabidopsis* CDPK gene family was analyzed and the expression, regulation, and possible functions of plant CDPKs was reviewed. By combining emerging cellular and genomic technologies with genetic and biochemical approaches, the characterization of *Arabidopsis* CDPKs provides a valuable opportunity to understand the plant calcium-signaling network (Cheng et al., 2002). These type of genes are Important for stress signaling.

***At2g25970 showing homology to KH RNA binding domain***

Lorkovic and Barta (2002) described RNA recognition motif (RRM) and K homology (KH) domain RNA-binding proteins from the flowering plant *Arabidopsis* thaliana. The most widely spread motifs are the RNA recognition motif (RRM) and the K homology (KH) domain. The *Arabidopsis* genome encodes 196 RRM-containing proteins, a more complex set than found in *Caenorhabditis elegans* and *Drosophila melanogaster*. In addition, the *Arabidopsis* genome contains 26 KH domain proteins. Most of the *Arabidopsis* RRM-containing proteins can be classified into structural and/or functional groups, based on similarity with either known metazoan or *Arabidopsis* proteins. Approximately 50% of *Arabidopsis* RRM-containing proteins do not have obvious homologues in metazoa, and for most of those that are predicted to be orthologues of metazoan proteins, no experimental data exist to confirm this. Additionally, the function of most *Arabidopsis* RRM proteins and of all KH proteins is unknown. The higher complexity of RNA-binding proteins in *Arabidopsis*, as evident in groups of SR splicing factors and poly(A)-binding proteins, may account for the observed differences in mRNA maturation between plants and metazoa. The function of this type of genes is largely unknown, but could

be related to PUMILIO genes from *Drosophila*. Important for regulation of gene expression at the post-transcriptional level, role in development, stress tolerance.

***At3g07800 showing homology to Thymidine kinase***

This type of thymidine kinase genes is cell cycle regulated, E2F regulated, is responsible for production of thymidine triphosphate. This type of gene plays a role as a precursor for DNA synthesis and is therefore a marker of S phase.

***At5g47370 showing homology to Homeobox leucine zipper protein*** .

This type of homeobox genes is important for development and growth and also for stress tolerance. At5g47370 is homeobox-leucine zipper protein HAT2 (HD-ZIP protein 2). Homeobox genes are known as transcriptional regulators that are involved in various aspects of developmental processes in many organisms. Homeodomain transcription factors regulate fundamental body plan of plants during embryogenesis, as well as morphogenetic events in the shoot apical meristem (SAM) after embryogenesis. HOX1 belongs to the subset of homeodomain leucine zipper (HD-zip) and is involved in the regulation of vascular development (Scarpella *et al.*, 2000; Meijer *et al.*, 2000). The sequences for the rice OsHOX1 orthologue are deposited in Genbank under the accession number X96681 (cDNA) and CAA65456.2 (protein), which sequences are both herein incorporated by reference.

***BAA23337.1 OsMYB1***

MYB-like DNA binding proteins are involved in the control of specific developmental steps in different organs. OSMYB1 binds to a seed specific element in the seed storage protein glutelin, is expressed in endosperm of rice seeds, and plays an important role during seed maturation (Suzuki *et al.*, 1997).

***BAA89798 OsNAC4***

NAC domain containing genes, such as NO APICAL MERISTEM in petunia and CUP-SHAPED COTYLEDON2 and NAP in *Arabidopsis*, have crucial functions in plant development (Kikuchi *et al.*, 2000). These genes are involved in the control of organ primordium delimitation and lateral organ development. It has also been recently shown that a member of the NAC family of transcription factor can induces formation of ectopic shoots on cotyledons (Daimon *et al.*, 2003).

***AAD37699 rice homeodomain leucine zipper protein HOX6 (partial)***

Homeobox genes are known as transcriptional regulators that are involved in various aspects of developmental processes in many organisms. Homeodomain transcription factors regulate

fundamental body plan of plants during embryogenesis, as well as morphogenetic events in the shoot apical meristem (SAM) after embryogenesis. HOX6 is a homologue of the *Arabidopsis* homeobox gene Athb-12 (Lee *et al.*, 2001). Athb-12 is a transcriptional activator important in regulating certain developmental processes as well as in the plant's response to water stress involving ABA-mediated gene expression. At3g61890 is the *Arabidopsis* sequence corresponding with the rice HOX6 sequence of AAD37699.

***AK104073 OsMYB predicted***

This gene is homologous to the *Arabidopsis* gene CIRCADIAN CLOCK ASSOCIATED (CCA1) gene that encodes a related MYB transcription factor, which regulates circadian rhythms (Carre et Kim, 2002). This gene as well as the MYB homologue, regulate the period of circadian rhythms in gene expression and leaf movements.

***Example 9: NMR study of E2Fa/DPa overexpressing plants.***

In support of the microarray studies identifying the increased or decreased expression level of E2F-target genes in E2Fa-DPa overexpressing plants, the effects of E2Fa/DPa overexpression on the protein level and ultimately on the level of metabolites were studied via the techniques of metabolomics. Metabolomics means qualitative and quantitative analysis of the metabolites present at a certain time in a cell culture or a whole biological tissue. Metabolites, as designated here, are small molecular weight molecules (typically under 1000 Daltons), of which many are already known (such as urea, lipids, glucose or certain small hormones) while others are still to be identified. Metabolites are the final product of the protein content of the cell. The main methods used to detect and quantify of those molecules are mass spectrometry or NMR spectroscopy (Nicholson *et al.*, 2002) after extraction and purification of the metabolites from the organism.

Now NMR spectroscopy on whole organisms has been performed. The recording of spectra of the metabolites was possible without any prior purification of the plant material. Hereto, the samples were spun at the magic angle. This technique, dubbed "High Resolution Magic Angle Spinning" (HRMAS) NMR, has now been used on intact plantlets. 1H-13C HSQC spectra were recorded on intact wild-type and E2Fa/DPa overexpressing plantlets of *Arabidopsis thaliana*, and monitored the changes in metabolite pattern. From the spectra, a shift in the metabolome of E2Fa/DPa overexpressing plants when compared to wild-type plants, was observed. These spectra are processed in order to map the observed metabolic differences.

***Example 10: molecular and phenotypic analysis of Arabidopisis plants transformed with the genes according to the present invention***

*Arabidopsis thaliana* plants are transformed with at least one of the genes of the present invention as presented in Table 4 or 5, operably linked to a plant promoter.

In one example, *Arabidopsis* plants were transformed with the genes as presented in Table 6. The vectors used ware derived from the expression vector pK7WGD2, carrying the CaMV35S promoter for expression of the gene. For transformation, the flower dip method described by Bechtold and Pelletier (1998) was used.

**Table 6: Genes that were selected and transformed into *Arabidopsis***

| CODE | AGI | GENE | PRIMERS | PCR | pDON R207 | PK7WGD2 | Flower dip |
|---|---|---|---|---|---|---|---|
| 1 | At1g33960 | AIG1 | 282 + 283 | | | | |
| 2 | At1g21690 | Putative replication factor | 284 + 285 | OK | OK | | |
| 3 | At3g23250 | Myb transcription factor | 286 + 287 | | | | |
| 4 | At5g08450 | Unknown | 288 + 289 | OK | OK | OK(clone1) | OK |
| 5 | At3g45730 | Unknown | 290 + 291 | OK | OK | OK(clone4) | OK |
| 6 | At1g56150 | Unknown | 292 + 293 | | | | |
| 7 | At5g66580 | Unknown | 294 + 295 | OK | OK | OK | OK |
| 8 | At4g33050 | Unknown | 296 + 297 | OK | OK | OK | OK |
| 9 | At1g76970 | Unknown | 298 + 299 | OK | partieel | OK(clone4) | |
| 10 | At2g41780 | Unknown | 300 + 301 | OK | OK | OK(clone1) | OK |
| 11 | At5g14530 | WD40 repeat protein | 302 + 303 | OK | OK | OK | OK |
| A | At3g02550 | Unknown | 310 + 311 | OK | OK | OK(A10.7) | OK |
| B | At5g47370 | homeobox-leucine zipper protein-like | 312 + 313 | OK | OK | OK(clone4) | OK |
| C | At1g57680 | Unknown | 314 + 315 | | | | OK |
| D | At1g07000 | leucine zipper-containing protein | 316 + 317 | OK | OK | | OK |
| E | At2g22430 | homeodomain TF Athb-6 | 318 + 319 | OK | OK | OK(clone1) | OK |
| F | At4g28330 | Unknown | 320 + 321 | OK | OK | OK(clone4) | OK |
| G | At3g23750 | receptor kinase | 322 + 323 | OK | OK | | |
| H | At5g66210 | Ca-dep kinase | 324 + 325 | OK | OK | OK(clone2) | OK |
| I | At4g02680 | Unknown | 326 + 327 | | | OK(clone4) | OK |
| J | At2g30590 | worky74 | 328 + 329 | OK | OK | OK(clone2) | OK |
| K | At2g46650 | Unknown | 330 + 331 | | | | |
| L | At2g47440 | Unknown | 332 + 333 | OK | OK | OK(clone5) | OK |
| M | At2g15510 | Unknown | 334 + 335 | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | At5g56740 | Histone acetylase HAT B | 348 + 349 | OK | OK | OK | OK |
| 13 | At3g24320 | Putative mismatch binding protein | 350 +351 | OK | OK | OK (13,4) | OK |
| 14 | At4g00730 | Anthocyaninless2 | 352 + 353 | | | | |
| 15 | At1g23030 | arm-repeat containing protein | 354 + 355 | OK | OK | OK(clone11) | OK |
| 16 | At5g54380 | receptor-protein kinase-like protein | 356 + 357 | | | | |
| 17 | At1g72180 | putative leucine-rich receptor-like protein kinase | 358 + 359 | OK | OK | OK | OK |
| 18 | At1g61100 | Unknown | 360 + 361 | OK | OK | OK (18,1) | OK |
| 19 | At2g25970 | Unknown | 362 + 363 | OK | OK | OK | OK |
| 20 | At2g38310 | Unknown | 364 + 365 | OK | OK | OK | OK |
| 21 | At3g45970 | Unknown | 366 + 367 | OK | OK | OK (21,3) | OK |

*Code: internal reference code of the gene; AGI: accession number of the protein in the internal dataset, here with reference to the MIPS database accession number; Gene: name of the protein; primers: PCR primers used to isolate the ORF of the gene by RT-PCR using cDNA; prepared form E2Fa-DPa overexpressing plants; PCR: PCR completed successfully; pDONR207: cloning in this vector completed (www.invitrogen.com); pK7WGD2: cloning of the genes in the vector under control of the CaMV 35S promoter (Karimi et al., Trends Plant Sci. 2002 May;7(5):193-5); Flower dip: transformation of Arabidopsis plants with the pK7WGD2 vector.*

The transformed *Arabidopsis* plants are evaluated as described below.
After molecular analysis (PCR, RT-PCR, Western-blot, southern-blot, Northern blot, NMR), the plants with modified E2F target gene expression levels, are submitted to phenotypic analysis. Special attention is given to root growth and leaf development.

The root of *A. thaliana*, which has a rather constant diameter and rather uncomplicated radial symmetry, is a perfect model system for studying and determining the effects of modulation of expression levels of an E2F-target on an intact, growing tissue.

The root of *A. thaliana* comprises a thick unicellular layer of the epidermis cells, one of cortex cells, one of endodermis cells and one of pericyclus cells that circumvent the vascular tube. Because of its transparency, the root of *A. thaliana*, these cellular layers can be visualized by interference contrast microscopy. By this means the origin of the cells in a specific cell layer can be traced back to a set of dividing mother cells in the meristem (Dolan *et al.*, 1993). By measurement of the cell length of a specific cell layer in function of the distance to the root tip,

and the rate of movement of the cells away from the root tip (measured via time-laps photography), it is possible to determine the contribution of both the cell elongation as well as of cell division to the total root growth (Beemster and Baskin, 1998).

The effects of the E2F-target overexpression in the leaves is determined via microscopic techniques after clearance of the leaves of lactic acid. This analysis is performed on the first developed leaf pear, since this leaf pear is most comparable between different plants. By measurement of the cell number and the number of epidermal cells at different time points during leaf development, it is deduced when the leaf cells stop to divide, when they start to differentiate, the duration of their cell cycle is, and their final cell size (De Veylder *et al.*, 2001 a and b). Moreover, this method allows the analysis of the effect of E2F target overexpression on the formation of stomata.

The effect of the E2F-target overexpression is also studied via biochemical means. Functional assays are developed for the specific enzymatic activity of the studied E2F-target gene. These functional methods are based on expression of a reported gene in case the E2F-target is in itself a transcription activator or repressor. Functional assays are based on the incorporation of radioactive nitrogen or radioactive carbon or other radiolabelled metabolites when the enzyme is involved in the nitrogen or carbon metabolisms or other processes involving metabolites. By the comparison of the incorporated radioactivity between the control line and the transgenic line, the enzymatic activity of the E2F-target can be measured.

Functional assays are based on the incorporation of radioactive ATP, radioactive purines or pyrimidines when the enzyme is involved in DNA replication and/or modification. Functional assays are based on labeled carbohydrates when the enzyme is involved in cell wall biogenesis, or ATP when the enzyme is involved in processes of the chloroplast, or calcium when the enzyme is involved in signal transduction.

In *A. thaliana,* besides the mitotic cell cycle also an alternative cell cycle is observed, in which DNA is replicated in the absence of mitosis or cytokinesis. This so-called endoreduplication process occurs often in plants. Until today, the physiological significance of endoreduplication is unknown. Possibly, it is a mechanism to increase the number of DNA copies per cell, which allows more transcription. In support of this hypothesis, endoreduplication often occurs in cells with high metabolic activity (Nagl, 1976). However, as a consequence of endoreduplication the cells are bigger, which is especially useful for increasing yield of cytoplasmatic component, for example storage proteins of the seed cells.

To study the effects of E2F-target overexpression on the process of endoreduplication, the DNA content of the control plants and the transgenic plant is measured via flow-cytometry. A more detailed analysis is obtained by measuring the DNA content of individual cells colored with DNA-binding fluorochrome (e.g. DAPI). The intensity of the color of the nucleus is in proportion with its DNA content. Relative DNA-measurements can be obtained via a microdensitometer. This technique allows determining a specific tissue the endoreduplication pattern of the transgenic plants.

***Example 11: Use of the invention in corn***

The invention described herein can also be used in maize. To this aim, a gene according to the present invention as presented in Table 4 or 5, for example a gene selected from Tables 1 or 2, or a gene selected from the group described in Example 8, or a gene selected from the group presented in Table 6 or 7, or a homologue thereof such as for example a maize ortholog or a rice ortholog, is cloned under control of a promoter operable in maize, in a plant transformation vector suited for *Agrobacterium*-mediated transformation of corn. These constructs are designed for overexpression or for downregulation. In a series of experiments, genes selected from Table 5 (downregulated in E2Fa/DP transgenics) are overexpressed in transgenic corn and genes selected from Table 4 (upregulated in E2Fa-DPa overexpressing plants) are downregulated in transgenic corn. Suitable promoter for driving expression of the genes of the present invention are as presented in Tables I, II, III and IV or in Table V.

Suitable promoter for driving expression of the genes of the present invention in corn are the rice GOS2 promoter or any other promoter as mentioned herein above. Vectors useful for expression of one or more E2F targets according to the present invention are standard binary vectors, such as the pPZP vector described in Hajdukiewicz *et al.* ((1994) Plant Mol Biol 25: 989-994) or a superbinary vector. Vectors and methods to use *Agrobactorium*-mediated transformation of maize have been described in literature (Ishida *et al.*, Nat Biotechnol. 1996 Jun;14(6):745-50; Frame *et al.*, Plant Physiol. 2002 May;129(1):13-22) and are herein incorporated by reference. Transgenic plants made by these methods are grown in the greenhouse for T1 seed production. Inheritability and copy number of the transgene are checked by quantitative real-time PCR and Southern blot analysis and expression levels of the transgene are determined by reverse PCR and Northern analysis. Transgenic lines with single copy insertions of the transgene and with varying levels of transgene expression are selected for T2 seed production. Progeny seeds are germinated and grown in the greenhouse in conditions well adapted for maize (16:8 photoperiod, 26-28°C daytime temperature and 22-24°C nighttime temperature) as well under water-deficient, nitrogen-deficient, and excess NaCl conditions. Null segregants from the same parental line, as well as wild type plants of the same

cultivar are used as controls. The progeny plants resulting from the selfing or the crosses are evaluated on different growth parameters, such as biomass and developmental parameters. These parameters include stem size, number of leaves, total above ground area, leaf greenness, time to maturity, flowering time, time to flower, ear number, harvesting time. The seeds of these lines are also checked on various parameters, such as grain size, total grain yield (number and/or weight) per plant, and grain quality (starch content, protein content and oil content). Lines that are most significantly improved versus the controls for any of the above-mentioned parameters are selected for further field-testing and marker-assisted breeding, with the objective of transferring the field-validated transgenic traits into commercial germplasm. Methods for testing maize for growth and yield-related parameters in the field are well established in the art, as are techniques for introgressing specific loci (such as transgene containing loci) from one germplasm into another. Corn plants according to the present invention have changed growth characteristics compared to the wild-type plants, such as for example any one or more of increased biomass, increased yield, increased number and/or size of organs (including seeds), increased harvest index, increased rate of growth and/or development (e.g. decreased cycling time, decreased time to harvest, early flowering), increased tolerance to environmental stress conditions (e.g. tolerance to salt, drought and/or cold).

***Example 12: Rice transformation with the genes according to the present invention***

In a particular example of the present invention, the genes as identified above in Tables 4 and 5, or an orthologue from another plant, for example the rice orthologue, is transformed into rice. In particular, the genes as presented in Tables 6 and 7, or the rice orthologues are cloned into a plant expression vector operably linked to a promoter for overexpression or downregulation of these genes.

The genes as represented in Table 7 are cloned into a plant expression vector operably linked to a GOS2 promoter for overexpression or downregulation. For overexpression these genes are cloned in the sense orientation and for downregulation a hairpin construct as described in Wesley *et al.* (2001) is made. Other promoters that are used to drive expression of these genes are other constitutive promoters, such as for example the ubiquitin promoter or PRO170 (high mobility group protein), or PRO61 (beta expansin promoter). Also tissue specific promoters are used to drive expression of the genes of the present invention in rice, such as for example promoters specific for meristem (PRO120: metallothionein), or vegetative tissue (PRO123: protochlorophyllid reductase), PRO173: cytoplasmic malate deshydrogenase); or endosperm (PRO90: prolamin, PRO135: alpha globulin), or embryo PRO218: oleosin, PRO151: WSI18, PRO200: OSH1, PRO175: RAB21; or the whole seed (PRO58: proteinase

inhibitor), or any other promoter described herein above. The vectors used are plant transformation vector suited for *Agrobacterium*-mediated transformation of rice, such as for example binary vectors of the pCAMBIA type or super binary vectors. Such vectors and methods for rice transformation have been described in literature by Aldemita and Hodges (1996) Chan *et al.* (1993), Hiei *et al.* (1994) or in EP1198985 and which teachings herein incorporated by reference.

**Table 7: genes (presented by their encoded proteins) selected for rice transformation**

| | |
|---|---|
| >CDS3435 NP_176081.1 At1g57680 (*Arabidopsis*)<br>MPLTKLVPDAFGVVTICLVALLVLLGLLCIAYSFYFQSHVRKQGYIQLGY<br>FSGPWIIRITFILFAIWWAVGEIFRLSLLRRHRRLLSGLDLRWQENVCKW<br>YIVSNLGFAEPCLFLTLMFLLRAPLKMESGALSGKWNRDTAGYIILYCLP<br>MLALQLAVVLSESRLNGGSGSYVKLPHDFTRTYSRVIIDHDEVALCTYP<br>LLSTILLGVFAAVLTAYLFWLGRQILKLVINKRLQKRVYTLIFSVSSFLPLR<br>IVMLCLSVLTAADKIIFEALSFLAFLSLFCFCVVSICLLVYFPVSDSMALRG<br>LRDTDDEDTAVTEERSGALLLAPNSSQTDEGLSLRGRRDSGSSTQERY<br>VELSLFLEAEN | SEQ ID NO 1<br>and 2 |
| >CDS3436 BAC42858.1 At3g45730 (*Arabidopsis*)<br>MELPSPYSSRKEESTVPPKRGRVKIMIFRDLVRSETSMAPTPRRGRIKK<br>MIAGDLVGSGKQNNYDGDGKRGG | SEQ ID NO 3<br>and 4 |
| >CDS3449 BAA23337.1 OS MYB1 (Rice)<br>MGRSPCCEKAHTNKGAWTKEEDQRLIAYIRAHGEGCWRSLPKAAGLL<br>RCGKSCRLRWMNYLRPDLKRGNFTDDEDELIIRLHSLLGNKWSLIAGQL<br>PGRTDNEIKNYWNTHIKRKLLARGIDPQTHRPLLSGGDGIAASNKRHHR<br>RRIPYPSRRRRPRRSSPCEAAAAAAPGRLLGRRLPQQQRHNEHGGA<br>AVPRPQPRALGRADAELAAGGDAHQRAAGLPLLPPRLPRRGGVQLSG | SEQ ID NO 5<br>and 6 |
| >CDS3448 BAA89798.1 OsNAC4 (rice)<br>MAAAVGGSGRRDAEAELNLPPGFRFHPTDEELVVHYLCRKVARQPLP<br>VPIIAEVDLYKLDPWDLPEKALFGRKEWYFFTPRDRKYPNGSRPNRAA<br>GRGYWKATGADKPVAPKGSARTVGIKKALVFYSGKAPRGVKTDWIMH<br>EYRLADADRAPGGKKGSQKLDEWVLCRLYNKKNNWEKVKLEQQDVA<br>SVAAAAPRNHHHQNGEVMDAAAADTMSDSFQTHDSDIDNASAGLRHG<br>GCGGGGFGDVAPPRNGFVTVKEDNDWFTGLNFDELQPPYMMNLQHM<br>QMQMVNPAAPGHDGGYLQSISSPQMKMWQTILPPF | SEQ ID NO 7<br>and 8 |
| >CDS3447 AAD37699.1 OS Homeodomain leucine zipper protein HOX6 (rice)<br>MDGEEDSEWMMMDVGGKGGKGGGGGGAADRKKRFSEEQIKSLESM | SEQ ID NO 9<br>and 10 |

| | |
|---|---|
| FATQTKLEPRQKLQLARELGLQPRQVAIWFQNKRARWKSKQLEREYSA LRDDYDALLCSYESLKKEKLALIKQLEKLAEMLQEPRGKYGDNAGDDA RSGGVAGMKKEEFVGAGGAATLYSSAEGGGTSSTEQTCSSTPWWEF ESE | |
| >CDS3446 AK104073 OSMYB predicted (rice)<br>MASIVTATVAAASAWWATQGLLPLFPPPIAFPFVPAPSAPFSTADVQRA QEKDIDCPMDNAQKELQETRKQDNFEAMKVIVSSETDESGKGEVSLHT ELKISPADKADTKPAAGAETSDVFGNKKKQDRSSCGSNTPSSSDIEAD NAPENQEKANDKAKQASCSNSSAGDNNHRRFRSSASTSDSWKEVSE EGRLAFDALFSRERLPQSFSPPQVEGSKEISKEEEDEVTTVTVDLNKNA AIIDQELDTADEPRASFPNELSNLKLKSRRTGFKPYKRCSVEAKENRVP ASDEVGTKRIRLESEAST | SEQ ID NO 11 and 12 |
| >CDS3445 NP_565887.1 At2g38310 (*Arabidopsis*)<br>MLAVHRPSSAVSDGDSVQIPMMIASFQKRFPSLSRDSTAARFHTHEVG PNQCCSAVIQEISAPISTVWSVVRRFDNPQAYKHFLKSCSVIGGDGDNV GSLRQVHVVSGLPAASSTERLDILDDERHVISFSVVGGDHRLSNYRSVT TLHPSPISGTVWESYVVDVPPGNTKEETCDFVDVIVRCNLQSLAKIAEN TAAESKKKMSL | SEQ ID NO 13 and 14 |
| >CDS3444 NP_565703.1 At2g30590WRKY family transcription factor (*Arabidopsis*)<br>MEEIEGTNRAAVESCHRVLNLLHRSQQQDHVGFEKNLVSETREAVIRF KRVGSLLSSSVGHARFRRAKKLQSHVSQSLLLDPCQQRTTEVPSSSSQ KTPVLRSGFQELSLRQPSDSLTLGTRSFSLNSNAKAPLLQLNQQTMPP SNYPTLFPVQQQQQQQQQQQQEQQQQQQQQQQFHERLQAHHL HQQQQLQKHQAELMLRKCNGGISLSFDNSSCTPTMSSTRSFVSSLSID GSVANIEGKNSFHFGVPSSTDQNSLHSKRKCPLKGDEHGSLKCGSSSR CHCAKKRKHRVRRSIRVPAISNKVADIPPDDYSWRKYGQKPIKGSPYPR GYYKCSSMRGCPARKHVERCLEDPAMLIVTYEAEHNHPKLPSQAITT | SEQ ID NO 15 and 16 |
| >CDS3443 NP_849867.1 At1g69510 (*Arabidopsis*)<br>MEDVKGKEIIDDAPIDNKVSDEMESEENAIKKKYGGLLPKKIPLISKDHE RAFFDSADWALGKQKGQKPKGPLEALRPKLQPTPQQQPRARRMAYSS GETEDTEIDNNEAPDDQACASAVDSTNLKDDGGAKDNIKS | SEQ ID NO 17 and 18 |
| >CDS3442 NP_564615.3 At1g52870 (*Arabidopsis*)<br>MAAASLHTSISPRSFLPLSKPSLKPHRSQILLRNKQRNCVSCALIRDEID LIPVQSRDRTDHEEGSVVVMSTETAVDGNESVVVGFSAATSEGQLSLE GFPSSSSSGADLGDEKRRENEEMEKMIDRTINATIVLAAGSYAITKLLTI DHDYWHGWTLFEILRYAPQHNWIAYEEALKQNPVLAKMVISGVVYSVG | SEQ ID NO 19 and 20 |

| | |
|---|---|
| DWIAQCYEGKPLFEIDRARTLRSGLVGFTLHGSLSHFYYQFCEELFPFQ<br>DWWVVPVKVAFDQTVWSAIWNSIYFTVLGFLRFESPISIFKELKATFLPM<br>LTAGWKLWPFAHLITYGLVPVEQRLLWVDCVELIWVTILSTYSNEKSEA<br>RISESVIETSSSSTTTIDPSKE | |
| >CDS3441 NP_849293.1 At4g02920 (*Arabidopsis*)<br>MIKLCFMTSHGYSIPGLGLPQDLCNTEIIKQNSRSHLVNPGARQEIIPAS<br>SFNLNTELLEPWKPVSSFSQFVEIDSAMMKPLLMDVHETAPESLILSFGI<br>ADKFARQEKVMEFLLSQSEEFKEKGFDMSLLNELMEFESMKSSSQLRP<br>YDTSSVLYLNQELGKPVLDLVRDMMENPEFSVRSNGHVLFSSSSNPEL<br>NDLLSIASEFNLSRNSTTKWRQLSPLIPHFQRFESDVFTPAKLKAVTVLA<br>PLKSPEKSRLKSPRKHNTKRKAKERDLYKRNHLHAYESLLSLMIGNDH<br>RHKHTTVLSLQKSCGELSELLTQFSITAAGTGIAVLFSVVCSLASRRVPF<br>CANKFFDTGLGLSLVILSWAVNRLREVIVHVNRKANKPCSSLKDDEIINS<br>VERSMKEVYYRAATVIAVFALRFAC | SEQ ID NO 21<br>and 22 |
| >CDS3440 AAM91100.1 At1g45200 (*Arabidopsis*)<br>MSKTNMKFCNSYFLVDPTKASFLDLLLLLFSSNLTSARFIDSPPDTLKGF<br>RRSFASRWILALAIFLQKVLMLLSKPFAFIGQKLTYWLNLLTANGGFFNLI<br>LNLMSGKLVKPDKSSATYTSFIGCSDRRIELDEKINVGSIEYKSMLSIMA<br>SKISYESKPYITSVVKNTWKMDLVGNYDFYNAFQESKLTQAFVFKTSST<br>NPDLIVVSFRGTEPFEAADWCTDLDLSWYEMKNVGKVHAGFSRALGL<br>QKDGWPKENISLLHQYAYYTIRQMLRDKLGRNKNLKYILTGHSLGGALA<br>ALFPAILAIHGEDELLDKLEGIYTFGQPRVGDEDFGEFMKGVVKKHGIEY<br>ERFVYNNDVVPRVPFDDKYLFSYKHYGPCNSFNSLYKGKVREDAPNA<br>NYFNLLWLIPQLLTGLWEFIRSFILQFWKGDEYKENWLMRFVRVVGIVF<br>PGGSNHFPFDYVNSTRLGGLVRPPPTTTPEDKLALIA | SEQ ID NO 23<br>and 24 |

Transgenic plants generated by these rice transformation methods are evaluated for various growth characteristics. More particularly, the transgenic plants are evaluated and the following parameters are monitored: increased total above ground biomass, increased plant height, increased number of tillers, increased number of first panicles, increased number of second panicles, increased total number of seeds, increased number of filled seeds, increased total seed yield (weigth) per plant, increased harvest index, increased thousand kernel weight, increased Tmid, increased T45 or A90, increased A42, changed cycling time or an changed growth curve, changed flowering time.

Plants with increase biomass, increased organ number and/or size (including seeds) and or any other economically attractive growth characteristics as found by the following plant

evaluation protocol, are selected to transferring the transgenic traits into commercial germplasm.

**Evaluation protocol for T0, T1 and T2 transgenic rice plants transformed with an E2F target gene according to the present invention**

Approximately 15 to 20 independent T0 rice transformants are generated. The primary transformants are transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Approximately 6 events, of which the T1 progeny segregated 3:1 for presence/absence of the transgene, are retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and approximately 10 T1 seedlings lacking the transgene (nullizygotes), are selected by monitoring screenable marker expression.

2 events with improved agronomical parameters in T1 are chosen for re-evaluation in T2 generation. Seed batches from the positive plants (both hetero- and homozygotes) in T1, are screened by monitoring marker expression. For each chosen event, the heterozygote seed batches are then selected for T2 evaluation. An equal number of positives and negatives within each seed batch are transplanted for evaluation in the greenhouse. The total number of 120 transformed plants is evaluated in the T2 generation. More particularly, two transformed events are selected, 60 plants per event of which 30 positives for the transgene, and 30 negative.

T1 and T2 plants are transferred to the greenhouse and evaluated for vegetative growth parameters and seed parameters, as described hereunder.

**Statistical analysis: t-test and F-test**

A two factor ANOVA (analysis of variants) is used as statistical model for the overall evaluation of plant phenotypic characteristics. An F-test is carried out on all the parameters measured, for all of the plants of all of the events transformed with the gene of interest. The F-test is carried out to check for an effect of the gene over all the transformation events and to determine the overall effect of the gene or "global gene effect". Significant data, as determined by the value of the F-test, indicates a "gene" effect, meaning that the phenotype observed is caused by more than the presence or position of the gene. In the case of the F-test, the threshold for significance for a global gene effect is set at a 5% probability level.

To check for an effect of the gene within an event, i.e., for a line-specific effect, a t-test is performed within each event using data sets from the transgenic plants and the corresponding null plants. "Null plants" or "Null segregants" are the plants treated in the same way as the transgenic plant, but from which the transgene has segregated. Null plants can also be described as the homozygous negative transformants. The threshold for significance for the

t-test is set at 10% probability level. Within one population of transformation events, some events can be under or above this t-test threshold. This is based on the hypothesis that a gene might only have an effect in certain positions in the genome, and that the occurrence of this position-dependent effect is not uncommon. This kind of gene effect may also be referred to as a "line effect of a gene". The p-value is obtained by comparing the t-value to the t-distribution or alternatively, by comparing the F-value to the F-distribution. The p-value stands for the probability that the null hypothesis (null hypothesis being "there is no effect of the transgene") is correct.

***Vegetative growth measurements:***

The selected transgenic plants are grown in a greenhouse. Each plant receives a unique barcode label to link unambiguously the phenotyping data to the corresponding plant. The selected transgenic plants are grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod= 11.5 h, daylight intensity= 30,000 lux or more, daytime temperature= 28°C or higher, night time temperature= 22°C, relative humidity= 60-70%. Transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. From the stage of sowing until the stage of maturity each plant is passed several times through a digital imaging cabinet and imaged. At each time point digital images (2048x1536 pixels, 16 million colours) are taken of each plant from at least 6 different angles. The parameters described below are derived in an automated way from all the digital images of all the plants, using image analysis software.

(a) Above ground plant area is determined by counting the total number of pixels from aboveground plant parts discriminated from the background. This value is averaged for the pictures taken on the same time point from the different angles and converted to a physical surface value expressed in square mm by calibration. Experiments show that the aboveground plant area measured this way correlates with the biomass of plant parts above ground.

(b) Plant height is determined by the distance between the horizontal lines going through the upper pot edge and the uppermost pixel corresponding to a plant part above ground. This value is averaged for the pictures taken on the same time point from the different angles and was converted, by calibration, to a physical distance expressed in mm. Experiments showed that plant height measured this way correlate with plant height measured manually with a ruler.

(C) Number of primary tillers is manually counted at the harvesting of the plants. The tillers are cut off at 3 cm above the pot rim. They were then counted at the cut surface. Tillers that were together in the same sheet were counted as one tiller.

(d) Number of primary panicles. The tallest panicle and all the panicles that overlap with the tallest panicles when aligned vertically are counted manually, and considered as primary panicles.

(e) Number of secondary panicles. The number of panicles that remained on the plant after the harvest of the primary panicles are counted and considered as secondary panicles.

(f) Growth curve. The plant area weekly measurements are modeled to obtain a growth curve for each plant, plotted as the value of plant area (in $mm^2$) over the time (in days). From this growth curve the following parameters are calculated.

(g) A42 is the plant area at day 42 after sowing as predicted by the growth curve model.

(h) Tmid is the time that a plant needs to grow and to reach 50% of the maximum plant area. Tmid is predicted from the growth curve model.

(i) T90 is the time that a plant needs to grow and to reach 90% of the maximum plant area. T90 is predicted from the growth curve model.

*Seed-related parameter measurements*

The mature primary panicles of T1 and T2 plants are harvested, bagged, barcode-labelled and then dried for three days in the oven at 37°C. The panicles are then threshed and all the seeds were collected and counted. The filled husks are separated from the empty ones using an air-blowing device. The empty husks are discarded and the remaining fraction is counted again. The filled husks are weighed on an analytical balance. This procedure resulted in the set of seed-related parameters described below.

(a) Total seed number per plant is measured by counting the number of husks harvested from a plant.

(b) Number of filled seeds is determined by counting the number of filled husks that remained after the separation step.

(c) Total seed yield per plant is measured by weighing all filled husks harvested from a plant.

(d) Harvest index of plants is defined as the ratio between the total seed yield and the above ground area ($mm^2$), multiplied by a factor $10^6$.

(e) Thousand Kernel Weight (TKW) of plants is a parameter extrapolated from the number of filled seeds counted, and their total weight.

(f) TotalArea Emergence Prop. is the time when plant reach 30 % of their maximum total area

(g) TotalArea Cycle Time. is the time when plant reach 90 % of their maximum total area

Further molecular analysis is performed on the positive plants by techniques well known by the person skilled in the art such as for example RT-PCR.

# Tables

## Table 1. Arabidopsis Genes 2-fold or more upregulated in E2Fa/DPa plants

| Gene Identification | accession # | MIPS name | OLD REF | | SEQ ID NO | | Fold induction | E2F site | Plant homologue |
|---|---|---|---|---|---|---|---|---|---|
| | | | cDNA | PROT | cDNA | PROT | | | |
| **Unknown function (14)** | | | | | | | | | |
| hypothetical protein | AI998042 | At1g57680 | 1 | 53 | 433 | 434 | 2.66 | | rice BAB90159.1, maize AY107220.1 |
| putative protein | AI994686 | At3g45730 | 2 | 54 | 231 | 232 | 5.14 | | |
| putative protein | AI994734 | At5g66580 | 4 | 56 | 489 | 490 | 3.18 | | |
| unknown protein | AI999397 | At2g38310 | 5 | 57 | 995 | 996 | 2.79 | TTTGCCCC | rice BAB68102.1 |
| unknown protein | AI995465 | At2g47440 | 7 | 59 | 931 | 932 | 2.50 | | |
| unknown protein | AI994871 | At1g76970 | 8 | 60 | 1193 | 1194 | 2.34 | | rice BAB78689.1, corn AAB00079.1 |
| hypothetical protein, kinesin | AI998366 | At1g27500 | 9 | 61 | 393 | 394 | 2.21 | | rice AAL87057.1 |
| putative protein | AI996967 | At4g33050 | 10 | 62 | 883 | 884 | 2.20 | | rice BAB90008.1 |
| putative protein | AI995917 | At3g43690 | 12 | 64 | 263 | 264 | 2.18 | | |
| unknown protein, kh domain protein | AI993084 | At2g25970 | 13 | 65 | 941 | 942 | 2.15 | | rice BAA92910.1, maize AY106526.1 |
| unknown protein | AI993077 | At1g68580 | 14 | 66 | 937 | 938 | 2.13 | | rice BAC00723.1, corn AAK11516.1 |
| putative protein, copine | AI993019 | At5g14420 | 15 | 67 | 205 | 206 | 2.05 | | rice BAB92575.1 |
| hypothetical protein | AI997428 | At1g57990 | 16 | 68 | 415 | 416 | 2.02 | | rice BAB90042.1 |
| unknown protein | AI997827 | At5g53740 | 17 | 69 | 2731 | 2732 | 2.01 | | |
| **DNA replication and modification (14)** | | | | | | | | | |
| putative thymidine kinase | AI997851 | At3g07800 | | | | | 8.44 | | rice AAC31168.1 |
| DNA methyltransferase | AI994691 | At5g49160 | | | | | 5.37 | ATTGCCGC | rice AAL77415.1, corn AAC16389.1 |
| Msi3 | AW004204 | At4g35050 | | | | | 4.89 | TTTCCCGC | corn AAL33648.1 |
| putative linker histone protein | AI994590 | At3g18035 | | | | | 3.31 | | |
| putative replication factor c | AI997934 | At1g21690 | | | | | 3.30 | TTTCCCGC | |
| topoisomerase 6 subunit A | AI995290 | At5g02820 | | | | | 2.62 | TTTCCCGC | |
| histone H4-like protein | AI999171 | At3g46320 | | | | | 2.55 | TTTGGCGC | |
| histone acetylase HAT B | AI998229 | At5g56740 | | | | | 2.36 | TTTCCCGC | corn AAM28228.1 |
| putative histon H1 | AI996137 | At1g06760 | | | | | 2.27 | | |
| histone H2A-like protein | AI995882 | At4g27230 | | | | | 2.23 | | |
| putative DNA gyrase subunit A | AI995400 | At3g10690 | | | | | 2.20 | | rice AAD29710.1 |
| histone H2B-like protein | AI999101 | At5g59910 | | | | | 2.16 | | |
| putative mismatch binding protein | AI993280 | At3g24320 | | | | | 2.10 | | rice CAD41187.1, corn AAF35250.1 |
| adenosylhomocysteinase | AI996953 | At4g13940 | | | | | 2.07 | | corn AAL33588.1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cell Cycle (2)** | | | | | | | | | |
| E2Fa | AJ294534 | At2g36010 | | | | | 94.88 | | |
| CDKB1;1 | D10851 | At3g54180 | | | | | 2.60 | TTTCCCG C | |
| **Cell wall biogenesis (11)** | | | | | | | | | |
| xyloglucan endo-1;4-beta-D-glucanase (meri-5) | AI994459 | At4g30270 | | | | | 3.74 | | |
| putative glycosyl transferase | AI999244 | At1g70090 | | | | | 3.38 | | |
| alpha galactosyltransferase-like protein | AI998223 | At3g62720 | | | | | 3.26 | | |
| putative xyloglucan endotransglycosylase | AI999683 | At3g23730 | | | | | 2.85 | | rice CAD41426.1, corn CAB510059.1 |
| xyloglucan endo-1,4-beta-D-glucanase-like protein | AI998301 | At4g30280 | | | | | 2.74 | | |
| putative xyloglucan endotransglycosylase | AI994477 | At1g14720 | | | | | 2.51 | | |
| putative glycosyl transferase | AI999770 | At1g24170 | | | | | 2.39 | | |
| putative UDP-glucose glucosyltransferase | AI997288 | At1g22400 | | | | | 2.34 | TTTCCCG C | |
| putative glucosyltransferase | AI998872 | At2g15480 | | | | | 2.15 | | |
| peroxidase | AI994622 | At2g38380 | | | | | 2.11 | TTTCGCC C | |
| beta-1,3-glucanase-like protein | AI994681 | At3g55430 | | | | | 2.05 | | rice AAB37697.1, corn CAB96424.1 |
| **Chloroplastic genes (7)** | | | | | | | | | |
| large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase | N96785 | rbcL | | | 2713 | 2714 | 4.71 | | NP_051067 |
| ribosomal protein L33 | AI994194 | rpl33 | | | 2715 | 2716 | 3.54 | | NP_051080 |
| PSII I protein | AW004203 | psbI | | | 2717 | 2718 | 2.81 | | NP_051074 |
| ribosomal protein L2 | AW004266 | rpl2 | | | 2719 | 2720 | 2.61 | | NP_051099 |
| ATP-dependent protease subunit | AI997947 | clpP | | | 2721 | 2722 | 2.60 | | NP_051083 |
| cytochrome B6 | AI997102 | petB | | | 2723 | 2724 | 2.55 | | NP_051088 |
| ATPase epsilon subunit | AW004251 | atpE | | | 2725 | 2726 | 2.17 | | NP_051065 |
| **Mitochondrial genes (1)** | | | | | | | | | |
| 26S ribosomal RNA protein | AW004275 | orf107a | | | 2727 | 2728 | 2.87 | | NP_085475 |
| **Transcription factors (6)** | | | | | | | | | |
| LOB domain protien 41 | AI996685 | At3g02550 | 3 | 55 | 1109 | 1110 | 4.01 | | riceBAB92193.1 |
| WRKY transcription factor 21 | AI992739 | At2g30590 | | | | | 2.78 | TTTCCCC C | |
| GATA Zn-finger protein | AI995731 | At3g16870 | 6 | 58 | 2729 | 2730 | 2.75 | | maize AY072149 |
| Anthocyaninless2 | AI993655 | At4g00730 | | | | | 2.73 | TTTCCCC C | |
| leucine zipper- | AI995691 | At1g07000 | | | | | 2.43 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| containing protein | | | | | | | | | |
| homeodomain transcription factor (Athb-6) | AI999190 | At2g22430 | | | | | 2.30 | | rice CAA65456.2, corn CAB96424.1 |
| **Metabolism and biogenesis (11)** | | | | | | | | | |
| alcohol dehydrogenase | AI998773 | At1g77120 | | | | | 5.09 | | |
| putative isocistrate lyase | AI999168 | At3g21720 | | | | | 3.08 | | |
| protochlorophyllide reductase precursor | AI993342 | At4g27440 | | | | | 2.39 | | |
| suger transpoter like protein | AI997793 | At4g36670 | | | | | 2.27 | | rice AAK13147.1, corn AAF74568.1 |
| NADH-dependent glutamate synthase (GOGAT) | AI997600 | At5g53460 | | | | | 2.25 | | |
| nitrate reductase (NIA2) | AI996208 | At1g37130 | | | | | 2.15 | | |
| pectate lyase - like protein | AJ508995 | At3g54920 | | | | | 2.13 | | |
| putative sterol dehydrogenase | AI996340 | At2g43420 | | | | | 2.10 | | |
| glutamine synthetase root isozyme 1 (GS) | 161G19T7 | At1g66200 | | | | | 2.06 | | |
| monosaccharide transporter STP3 | AI997045 | At5g61520 | | | | | 2.05 | | rice BAA83554.1, corn AAF74568.1 |
| **Signal transduction (6)** | | | | | | | | | |
| calcium-dependent protein kinase | AI996555 | At5g66210 | | | | | 2.96 | | rice AAF23901.2, corn BAA12715.1 |
| WD-40 repeat protein | AI993055 | At5g14530 | | | | | 2.70 | | rice AD27557.1, corn AAA50446.1 |
| receptor-protien kinase-like protein | AI994727 | At5g54380 | | | | | 2.59 | | rice AAK63934.1, corn AAB09771.1 |
| putative phytochrome A | AI998146 | At1g09570 | | | | | 2.45 | | |
| putative leucine-rich receptor-like protein kinase | AI999651 | At1g72180 | | | | | 2.13 | | rice BAC06203.1, corn CAC35411.1 |
| putative receptor-like kinase | AI993298 | At3g23750 | | | | | 2.06 | | rice CAA69028.1, corn CAC35412.1 |
| **Others (13)** | | | | | | | | | |
| putative pollen allergen | AI996548 | At3g45970 | | | | | 3.22 | | rice AAG13596.1, corn CAD40849.1 |
| cold-regulated protein COR6,6 | AW004198 | At5g15970 | | | | | 3.03 | | |
| phi-1-like protein | AI994601 | At5g64260 | | | | | 2.60 | | |
| lipid-transfer protein-like | AI998609 | At5g01870 | | | | | 2.33 | | rice BAB86497.1, corn AAB06443.1 |
| DnaJ homologue | AI994551 | At5g06910 | | | | | 2.32 | ATTGGCGC | |
| blue copper binding protein | AI996535 | At5g20230 | | | | | 2.30 | | |
| src-2 like protein | AI998679 | At1g09070 | 11 | 63 | 401 | 402 | 2.19 | | |
| RING finger protein | AI999491 | At3g61460 | | | | | 2.14 | | rice BAA85438.1, corn AAL59234.1 |
| putative Ticc22 | AI993361 | At3g23710 | | | | | 2.14 | | |
| nodulin-like protein | AI996322 | At1g80530 | | | | | 2.07 | | rice AAM01022.1 |
| putative resistance protein | AI997549 | At1g61100 | | | | | 2.06 | | rice AAL83695.1, |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| seed imbitition protein-like | AI993446 | At5g20250 | | | | | 2.05 | | |
| putative disease resistance protein | AI998978 | At1g72900 | | | | | 2.04 | | rice AAL01163.1, corn AAC83564.1 |

## Table 2. Arabidopsis Genes 2-fold or more repressed in E2Fa/DPa plants

| Gene Identification | accession # | MIPS | OLD REF | | SEQ ID NO | | Fold repression | E2F site | plant hom ologue |
|---|---|---|---|---|---|---|---|---|---|
| | | | cDNA | PROT | cDNA | PROT | | | |
| **Unknown function (35)** | | | | | | | | | |
| unknown protein | AI993767 | At1g45200* | 18 | 70 | 2741 | 2742 | 3.91 | | |
| putative protein | AI993468 | At3g56290 | 19 | 71 | 1483 | 1484 | 3.38 | | maize AY106321.1 , rice BAB93184.1 |
| hypothetical protein, multidrug efflux protein | AI996374 | At1g61890 | 21 | 73 | 2599 | 2600 | 2.78 | | |
| unknown protein | AI994573 | At3g15950 | 22 | 74 | 2147 | 2148 | 2.71 | | |
| putative protein | AI994726 | At3g52360 | 23 | 75 | 1619 | 1620 | 2.65 | | |
| hypothetical protein | AI997393 | At4g02920 | 24 | 76 | 1521 | 1522 | 2.60 | TTTGCCCC | Y09602. Hordeum vulgare |
| unknown protein, put protease inhibitor | AJ508997 | At5g43580 | 25 | 77 | 2743 | 2744 | 2.58 | | |
| unknown protein | AI997866 | At1g70760 | 26 | 78 | 2077 | 2078 | 2.52 | | |
| unknown protein | AI997085 | At5g43750 | 27 | 79 | 1423 | 1424 | 2.51 | | rice BAB90754.1 |
| putative protein | AI995724 | At5g50100** | 28 | 80 | 1973 | 1974 | 2.48 | | rice AL606619.2 OSJN00032 genomic |
| unknown protein | AI995337 | At1g74880 | 29 | 81 | 2699 | 2700 | 2.42 | | maize AY105515.1, rice BAB89011.1 |
| unknown protein | AI998296 | At3g19370 | 30 | 82 | 1859 | 1860 | 2.40 | | |
| unknown protein, ATP ase | AI993346 | At3g10420 | 31 | 83 | 2249 | 2250 | 2.40 | | |
| putative protein | AI999485 | At3g61080 | 32 | 84 | 1863 | 1864 | 2.38 | | |
| unknown protein | AI996923 | At1g67860 | 33 | 85 | 1847 | 1848 | 2.38 | | |
| unknown protein | AI994841 | At1g52870 | 34 | 86 | 2367 | 2638 | 2.35 | ATTCCCCC | maize AY108423.1 |
| unknown protein | AI999581 | At1g64370 | 35 | 87 | 2099 | 2100 | 2.35 | | |
| unknown protein | AI997584 | At1g05870 | 36 | 88 | 1955 | 1956 | 2.25 | | rice BAB86085.1, maize Y110580.1 |
| putative protein | AI992938 | At5g03540 | 37 | 89 | 2745 | 2746 | 2.21 | | |
| hypothetical protein | AI997712 | At2g15020 | 38 | 90 | 2605 | 2606 | 2.21 | | rice BAB64794.1 |
| unknown protein | AI998338 | At1g68440 | 39 | 91 | 2625 | 2626 | 2.20 | | |
| unknown protein | AI996872 | At2g21960 | 40 | 92 | 1715 | 1716 | 2.19 | | |
| putative protein, centrin | AI996295 | At4g27280 | 41 | 93 | 2039 | 2040 | 2.18 | | |
| putative protein | AI995642 | At3g48200 | 42 | 94 | 2653 | 2654 | 2.16 | | |
| unknown protein | AI997470 | At2g32870 | 43 | 95 | 1941 | 1942 | 2.14 | | |
| hypothetical protein | AI998460 | At1g69510 | 44 | 96 | 2019 | 2020 | 2.11 | TTTGGCCC | rice BAB18340.1, maize AY110240.1 |
| putative triacylglycerol lipase | AI993356 | At5g22460 | 45 | 97 | 2349 | 2350 | 2.10 | | |
| putative protein | AI995956 | At5g52060 | 46 | 98 | 1779 | 1780 | 2.08 | | |
| unknown protein | AI996100 | At2g35830 | 47 | 99 | 2471 | 2472 | 2.06 | | |
| hypothetical protein | AI996039 | At3g27050 | 48 | 100 | 2175 | 2176 | 2.05 | | |
| unknown protein | AI996020 | At5g51720 | 49 | 101 | 2033 | 2034 | 2.04 | | |
| putative protein | AW004101 | At4g39730 | 51 | 103 | 1605 | 1606 | 2.03 | | |
| hypothetical protein | AI998372 | At2g01260 | 52 | 104 | 1979 | 1980 | 2.03 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| unknown protein | AI999573 | At3g61060 | | | | | 2.00 | | |
| unknown protein | AI998562 | At2g35760 | | | | | 2.00 | | |
| No hit (2) | | | | | | | | | |
| no hit on genome | AI995690 | | | | | | 2.54 | | |
| no hit on genome | AI999010 | | | | | | 2.23 | | |
| **Cell wall biogenesis (4)** | | | | | | | | | |
| similar to polygalacturonase-like protein | AI993509 | At1g10640 | 50 | 102 | 1761 | 1762 | 3.62 | | maize AY106712.1, rice BAC06884.1 |
| putative xyloglucan endo-transglycosylase | AI997647 | At2g36870 | | | | | 2.51 | | |
| pectate lyase 1-like protein | AI994801 | At1g67750 | | | | | 2.40 | | |
| xyloglucan endo-transglycosylase | AI998832 | At3g44990 | | | | | 2.35 | | |
| **Metabolism and biogenesis (24)** | | | | | | | | | |
| fructose-biphosphate aldolase-like protein | AI994456 | At4g26530 | | | | | 5.99 | ATTGGCCC | |
| sucrose-phosphate synthase-like protein | AI995432 | At4g10120 | | | | | 4.64 | | |
| putative branched-chain amino acid aminotransferase | AI997263 | At3g19710 | | | | | 3.31 | | |
| vitamine c-2 | AI997404 | At4g26850 | 20 | 72 | 2511 | 2512 | 3.04 | TTTGCCGC | maize AY105327, rice BAB90526.1 |
| nicotianamine synthase | AI993200 | At5g04950 | | | | | 2.86 | | |
| beta-fructosidase | AI994670 | At1g62660 | | | | | 2.66 | TTTCCCCC | |
| neoxanthin cleavage enzyme-like protein | AI997269 | At4g19170 | | | | | 2.66 | | |
| putative starch synthase | AI997174 | At1g32900 | | | | | 2.63 | | |
| cytochrome P450 monooxygenase (CYP83A1) | AI994017 | At4g13770 | | | | | 2.57 | | |
| beta-amylase-like protein | AI999322 | At5g18670 | | | | | 2.53 | | |
| FRO1-like protein; NADPH oxidase-like | AI995987 | At5g49740 | | | | | 2.46 | | |
| putative hydrolase | AI997149 | At3g48420 | | | | | 2.39 | | |
| furamate hydratase | AI997067 | At5g50950 | | | | | 2.31 | TTTGGCCC | |
| 5'-adenylylsulfate reductase | AI992757 | At1g62180 | | | | | 2.30 | TTTCCCCC | |
| 5'-adenylylsulfate reductase | AI996614 | At4g04610 | | | | | 2.30 | | |
| UDP rhamnose-anthocyanidin-3-glucoside rhamnosyltransferase -like protein | AI996803 | At4g27560 | | | | | 2.24 | | |
| cytochrome P450-like protein | AI993171 | At5g48000 | | | | | 2.23 | | |
| lactoylglutathione lyase-like protein | AI994552 | At1g11840 | | | | | 2.20 | | |
| putative beta-glucosidase | AI995306 | At4g27820 | | | | | 2.20 | ATTGGCCC | |

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|---|---|---|---|---|---|---|---|---|---|
| adenine phospho-ribosyltransferase-like protein | AI994567 | At4g22570 | | | | | 2.18 | | |
| catalase | AI995830 | At4g35090 | | | | | 2.17 | ATTCCCCC | |
| putative glutathione peroxidase | AW004143 | At2g25080 | | | | | 2.15 | | |
| putative adenosine phosphosulfate kinase | AW004219 | At2g14750 | | | | | 2.13 | | |
| tyrosine transaminase like protein | AI996914 | At4g23600 | | | | | 2.13 | | |
| **Transcription factors (5)** | | | | | | | | | |
| homeobox-leucine zipper protein ATHB-12 | AI994027 | At3g61890 | | | | | 4.20 | | |
| NAC domain protein NAC2 | AI992865 | At1g69490 | | | | | 3.68 | | |
| myb-related transcription factor | AI995298 | At1g71030 | | | | | 2.78 | | |
| dof zinc finger protein | AI994875 | At1g51700 | | | | | 2.30 | | |
| MYB-related transcription factor (CCA1) | AI992931 | At2g46830 | | | | | 2.19 | | |
| **Signal transduction (9)** | | | | | | | | | |
| serine/threonine protein kinase-like protein | AI995557 | At5g10930 | | | | | 3.91 | | |
| subtilisin proteinase-like | AI993428 | At4g21650 | | | | | 3.19 | | |
| putative oligopeptide transporter | AI996160 | At4g10770 | | | | | 2.68 | | |
| putative lectin | AI998542 | At3g16400 | | | | | 2.52 | | |
| Ca2+dependent membrane-binding protein annexin | AI998553 | At1g35720 | | | | | 2.45 | | |
| putative WD repeat protein | AI997238 | At3g15880 | | | | | 2.38 | | |
| putative lectin | AI999016 | At3g16390 | | | | | 2.35 | | |
| putative lectin | AI993358 | At3g16530 | | | | | 2.31 | | |
| SNF1 related protein kinase (ATSRPK1) | AI993111 | At3g23000 | | | | | 2.06 | | |
| **Others (25)** | | | | | | | | | |
| putative protease inhibitor Dr4 | AI995265 | At1g73330 | | | | | 10.30 | | |
| major latex protein homolog - like | AI998305 | At2g01520 | | | | | 4.27 | | |
| pollen allergen-like protein | AI993041 | At1g24020 | | | | | 3.56 | | |
| putative heat shock protein | AI997846 | At1g06460 | | | | | 3.55 | | |
| putative fibrillin | AI997199 | At4g04020 | | | | | 3.55 | | |
| major latex protein homolog - like | AI997255 | At1g70890 | | | | | 3.50 | | |
| putative nematode-resistance protein | AI993740 | At2g40000 | | | | | 2.95 | | |
| putative auxin-regulated protein | AJ508998 | At2g46690 | | | | | 2.86 | | |
| putative myrosinase-binding protein | AI997583 | At2g39310 | | | | | 2.61 | | |
| ubiquitin-conjugating enzyme-like protein | AI997782 | At5g56150 | | | | | 2.41 | | |

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|---|---|---|---|---|---|---|---|---|---|
| ubiquitin-conjugating enzyme E2-17 kD 8 | AI994771 | At5g41700 | | | | | 2.40 | | |
| vegetative storage protein Vsp2 | AI999152 | At5g24770 | | | | | 2.35 | | |
| heat shock protein 70 | AI994044 | At3g12580 | | | | | 2.24 | | |
| chloroplast outer envelope membrane protein | AI997015 | At3g63160 | | | | | 2.20 | | |
| translation initiation factor-like protein | AI992786 | At5g54940 | | | | | 2.15 | | |
| pseudogene | AI995323 | At2g04110 | | | | | 2.07 | | |
| vegetative storage protein Vsp1 | AI999546 | At5g24780 | | | | | 2.06 | | |
| dehydrin ERD10 | AI997518 | At1g20450 | | | | | 2.06 | | |
| MTN3-like protein | AI997159 | At3g48740 | | | | | 2.05 | | |
| putative chlorophyll A-B binding protein | AI994859 | At3g27690 | | | | | 2.05 | | |
| photosystem I reaction centre subunit psaN | AI997939 | At5g64040 | | | | | 2.03 | | |
| AR781, similar to yeast pheromone receptor | AI998194 | At2g26530 | | | | | 2.03 | | |
| putative lipid transfer protein | AI997024 | At2g15050 | | | | | 2.03 | | |
| peroxidase ATP3a | AI998372 | At5g64100 | | | | | 2.03 | | |
| myosin heavy chain-like protein | AI999224 | At3g16000 | | | | | 2.01 | | |

- * this sequence is present in the MIPs database version of 25 july 2002
- ** this record has an updated MIPS accession number At5g50101.

**Table 3. Number of E2F elements in the different datasets**

| | All genes (4518) | Upregulated genes (88) | Downregulated genes (105) |
|---|---|---|---|
| TTTCCCCC | 62 | 2 | 3 |
| TTTCCCGC | 40 | 6 | 0 |
| TTTCGCCC | 15 | 0 | 0 |
| TTTCGCCC | 13 | 1 | 0 |
| TTTGCCCC | 37 | 1 | 1 |
| TTTGCCGC | 20 | 0 | 1 |
| TTTGGCCC | 55 | 0 | 2 |
| TTTGGCGC | 15 | 1 | 0 |
| ATTCCCCC | 10 | 0 | 2 |
| ATTCCCGC | 6 | 0 | 0 |
| ATTCGCCC | 8 | 0 | 0 |
| ATTCGCCC | 14 | 0 | 0 |
| ATTGCCCC | 13 | 0 | 0 |
| ATTGCCGC | 10 | 1 | 0 |
| ATTGGCCC | 44 | 0 | 2 |
| ATTGGCGC | 9 | 1 | 0 |
| Total | 371 | 13 | 11 |

**Table 4: Arabidopsis genes 1.3 fold or more upregulated in E2Fa/Dpa plants**

| SEQ ID NO | | Gene name | e_value | MIPS accession number | ratio |
|---|---|---|---|---|---|
| cDNA | PROT | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 25 | 26 | putative protein | 0 | At5g51100 | 1.42 |
| 27 | 28 | endo-1,4-beta-glucanase | 9E-27 | At1g70710 | 1.85 |
| 29 | 30 | mitochondrial elongation factor Tu | 1E-125 | At4g02930 | 1.39 |
| 31 | 32 | glycine-rich protein (clone AtGRP8) | 1E-155 | At4g39260 | 1.33 |
| 33 | 34 | UTP-glucose glucosyltransferase | 0 | At5g66690 | 1.59 |
| 35 | 36 | lipid-transfer protein-like | 0 | At5g01870 | 2.33 |
| 37 | 38 | putative auxin-regulated protein | 6E-68 | At4g34760 | 1.48 |
| 39 | 40 | histone H1, putative | 0 | At1g06760 | 2.27 |
| 41 | 42 | APETALA2 protein | 0 | At4g36920 | 1.44 |
| 43 | 44 | putative histone H2A | 0 | At1g08880 | 1.84 |
| 45 | 46 | monosaccharide transporter STP3 | 2E-69 | At5g61520 | 2.05 |
| 47 | 48 | receptor-protein kinase-like protein | 8E-64 | At3g51550 | 1.33 |
| 49 | 50 | SET-domain protein-like | 1E-140 | At5g04940 | 1.38 |
| 51 | 52 | homeodomain transcription factor (ATHB-6) | 0 | At2g22430 | 2.3 |
| 53 | 54 | putative protein | 0 | At4g33700 | 1.85 |
| 55 | 56 | hypothetical protein | 1E-139 | At1g05800 | 1.34 |
| 57 | 58 | unknown protein | 0 | At1g33410 | 1.37 |
| 59 | 60 | hypothetical protein | 1E-140 | At4g17060 | 1.41 |
| 61 | 62 | putative protein | 0 | At5g19820 | 1.44 |
| 63 | 64 | putative protein | 1E+00 | At3g53670 | 1.54 |
| 65 | 66 | regulatory subunit of protein kinase CK2 | 0 | At3g60250 | 1.51 |
| 67 | 68 | delta 9 desaturase, putative | 0 | At1g06090 | 1.85 |
| 69 | 70 | putative protein | 0 | At5g06360 | 1.48 |
| 71 | 72 | acetyl-CoA carboxylase, putative, 5' partial | 0 | At1g36170*** | 1.49 |
| 73 | 74 | hypothetical protein | 0 | At1g56150 | 1.97 |
| 75 | 76 | seed imbitition protein-like | 0 | At5g20250 | 2.05 |
| 77 | 78 | unknown protein | 1E-146 | At1g76010 | 1.64 |
| 79 | 80 | homeobox-leucine zipper protein-like | 0 | At5g47370 | 2.21 |
| 81 | 82 | kinesin-like protein | 0 | At5g54670 | 1.69 |
| 83 | 84 | putative protein | 0 | At3g48050 | 1.75 |
| 85 | 86 | putative protein | 0 | At5g03040 | 1.34 |
| 87 | 88 | xyloglucan endo-1,4-beta-D-glucanase precursor | 0 | At4g30270 | 3.74 |
| 89 | 90 | putative WD-40 repeat protein | 0 | At2g19540 | 1.75 |
| 91 | 92 | putative protein | 1E-132 | At3g54480 | 1.44 |
| 93 | 94 | hypothetical protein | 0 | At1g15750 | 1.7 |
| 95 | 96 | hypothetical protein | 0 | At1g66200 | 2.06 |
| 97 | 98 | putative protein | 0 | At3g50630 | 1.4 |
| 99 | 100 | unknown protein | 0 | At2g30930 | 1.3 |
| 101 | 102 | putative protein | 6E-91 | At5g37720 | 1.8 |
| 103 | 104 | unknown protein | 1E-146 | At5g54310 | 1.61 |
| 105 | 106 | hypothetical protein | 0 | At1g48920 | 1.98 |
| 107 | 108 | hypothetical protein | 0 | At1g17750 | 1.38 |
| 109 | 110 | nuclear RNA binding protein A-like protein | 0 | At4g17520 | 1.43 |
| 111 | 112 | unknown protein | 4E+00 | At1g10890 | 1.38 |
| 113 | 114 | histone H2A- like protein | 0 | At4g27230 | 2.23 |
| 115 | 116 | phytochelatin synthase (gb\|AAD41794.1) | 0 | At5g44070 | 1.39 |
| 117 | 118 | RNA-binding protein cp29 protein | 1E-159 | At3g53460 | 1.54 |
| 119 | 120 | putative RNA-binding protein | 0 | At3g25150 | 1.48 |
| 121 | 122 | alcohol dehydrogenase | 2E-01 | At5g42250 | 1.34 |
| 123 | 124 | putative 60S ribosomal protein L6 | 1E-170 | At1g74060 | 1.37 |
| 125 | 126 | calmodulin-binding protein | 1E-114 | At5g57580 | 1.4 |
| 127 | 128 | putative protein | 3E-23 | At4g20310 | 2.01 |

| | | | | | |
|---|---|---|---|---|---|
| 129 | 130 | putative protein kinase | 0 | At1g08720 | 1.33 |
| 131 | 132 | hypothetical protein | 0 | At3g12200 | 1.34 |
| 133 | 134 | putative phosphatidylserine decarboxylase | 0 | At4g25970 | 1.38 |
| 135 | 136 | unknown protein | 0 | At2g03120 | 1.31 |
| 137 | 138 | unknown protein | 0 | At1g14880 | 1.48 |
| 139 | 140 | histone H2A.F/Z | 0 | At3g54560 | 1.85 |
| 141 | 142 | 4-coumarate-CoA ligase - like | 0 | At4g19010 | 1.35 |
| 143 | 144 | putative protein | 0 | At3g45040 | 1.72 |
| 145 | 146 | unknown protein | 0 | At3g19540 | 1.84 |
| 147 | 148 | putative protein | 0 | At4g34410 | 1.36 |
| 149 | 150 | unknown protein | 0 | At1g61260 | 1.97 |
| 151 | 152 | putative protein | 0 | At3g61490 | 1.32 |
| 153 | 154 | lipoxygenase | 0 | At1g17420 | 1.34 |
| 155 | 156 | putative SecA-type chloroplast protein transport factor | 0 | At4g01800 | 1.38 |
| 157 | 158 | putative DNA-binding protein | 0 | At4g01250 | 1.49 |
| 159 | 160 | hypothetical protein | 0 | At1g20580 | 1.37 |
| 161 | 162 | hypothetical protein | 2E-90 | At1g47530 | 1.39 |
| 163 | 164 | unknown protein | 0 | At2g37570 | 1.84 |
| 165 | 166 | bZIP transcription factor-like protein | 0 | At3g62420 | 1.32 |
| 167 | 168 | putative protein | 1E-154 | At3g56720 | 1.39 |
| 169 | 170 | hypothetical protein | 0 | At1g76860 | 1.32 |
| 171 | 172 | 6-phosphogluconate dehydrogenase | 2E-80 | At5g41670 | 1.48 |
| 173 | 174 | ferritin 1 precursor | 0 | At5g01600 | 1.38 |
| 175 | 176 | putative ABC transporter | 0 | At1g71330 | 1.71 |
| 177 | 178 | hypothetical protein | 0 | At1g27300 | 1.3 |
| 179 | 180 | myrosinase precursor | 9E-01 | At5g26000 | 2.81 |
| 181 | 182 | unknown protein | 0E+00 | At1g10270 | 1.47 |
| 183 | 184 | putative protein | 3E-88 | At5g18650 | 1.33 |
| 185 | 186 | hypothetical protein | 6E-40 | At2g36090 | 1.32 |
| 187 | 188 | unknown protein | 0 | At1g43910 | 1.42 |
| 189 | 190 | hypothetical protein | 0 | At1g07000 | 2.43 |
| 191 | 192 | hypothetical protein | 0 | At1g18260 | 1.43 |
| 193 | 194 | putative pre-mRNA splicing factor | 0 | At4g03430 | 1.49 |
| 195 | 196 | putative protein | 0 | At5g11810 | 1.32 |
| 197 | 198 | hypothetical protein | 1E-151 | At4g30150 | 1.41 |
| 199 | 200 | S-receptor kinase -like protein | 0 | At4g32300 | 1.52 |
| 201 | 202 | disease resistance RPP5 like protein | 1E-175 | At4g16950 | 1.64 |
| 203 | 204 | unknown protein | 2E-58 | At1g76520 | 1.44 |
| 205 | 206 | putative protein | 1E-144 | At5g14420 | 2.05 |
| 207 | 208 | putative glucosyltransferase | 4E-78 | At1g23480 | 1.31 |
| 209 | 210 | putative protein | 1E-144 | At4g28470 | 1.34 |
| 211 | 212 | putative protein | 0 | At4g29830 | 1.55 |
| 213 | 214 | putative auxin-regulated protein | 0 | At2g33830 | 1.41 |
| 215 | 216 | putative protein | 8E+00 | At5g61550 | 1.38 |
| 217 | 218 | unknown protein | 0 | At1g44810 | 1.39 |
| 219 | 220 | protein phosphatase - like protein | 1E-59 | At5g02760 | 1.76 |
| 221 | 222 | hypothetical protein | 2E-21 | At4g17800 | 1.59 |
| 223 | 224 | hypothetical protein | 0 | At1g54080 | 1.58 |
| 225 | 226 | xyloglucan endo-transglycosylase, putative | 0 | At1g14720 | 2.51 |
| 227 | 228 | putative protein | 0 | At3g49320 | 1.7 |
| 229 | 230 | beta-1,3-glucanase - like protein | 0 | At3g55430 | 2.05 |
| 231 | 232 | putative protein | 0 | At3g45730 | 5.14 |

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|---|---|---|---|---|---|
| 233 | 234 | ubiquitin-conjugating enzyme E2-21 kD 1 (ubiquitin-protein ligase) | 0 | At5g41340 | 1.32 |
| 235 | 236 | putative reticuline oxidase-like protein | 0 | At1g30720 | 1.31 |
| 237 | 238 | DNA (cytosine-5)-methyltransferase (DNA methyltransferase) (DNA | 0 | At5g49160 | 5.37 |
| 239 | 240 | putative protein | 0 | At4g32030 | 1.38 |
| 241 | 242 | unknown protein | 3E+00 | At2g32710 | 1.46 |
| 243 | 244 | E2F transcription factor-1 E2F1 | 1E-155 | At5g22220 | 1.52 |
| 245 | 246 | putative protein | 0 | At5g48820 | 1.8 |
| 247 | 248 | putative E2F5 family transcription factor | 1E-154 | At2g36010 | 94.9 |
| 249 | 250 | protein kinase cdc2 homolog B | 0 | At3g54180 | 2.6 |
| 251 | 252 | putative WRKY DNA-binding protein | 1E-164 | At2g03340 | 1.43 |
| 253 | 254 | hypothetical protein | 0 | At4g13670 | 1.56 |
| 255 | 256 | xyloglucan endo-1,4-beta-D-glucanase-like protein | 0 | At4g30280 | 2.74 |
| 257 | 258 | hypothetical protein | 1E-121 | At1g18630 | 1.41 |
| 259 | 260 | putative protein | 0 | At5g35735 | 1.52 |
| 261 | 262 | putative protein kinase | 0 | At2g47060 | 1.32 |
| 263 | 264 | putative protein | 1E-01 | At3g43690 | 2.18 |
| 265 | 266 | 70kD heat shock protein | 0 | At2g32120 | 1.57 |
| 267 | 268 | nitrate reductase | 0 | At1g37130 | 2.15 |
| 269 | 270 | beta-amylase | 0 | At5g55700 | 1.55 |
| 271 | 272 | multicatalytic endopeptidase complex alpha chain | 0 | At3g51260 | 1.57 |
| 273 | 274 | putative protein | 3E-02 | At5g36190 | 2.55 |
| 275 | 276 | putative protein | 0 | At4g00830 | 1.39 |
| 277 | 278 | monodehydroascorbate reductase (NADH) - like protein | 0 | At5g03630 | 1.33 |
| 279 | 280 | unknown protein | 1E-107 | At3g04350 | 1.42 |
| 281 | 282 | hypothetical protein | 0 | At1g70090 | 3.38 |
| 283 | 284 | E2 ubiquitin-conjugating-like enzyme Ahus5 | 0 | At3g57870 | 1.38 |
| 285 | 286 | putative protein | 5E-25 | At3g63070 | 1.35 |
| 287 | 288 | hypothetical protein | 0 | At4g28330 | 2.23 |
| 289 | 290 | cellulose synthase catalytic subunit, putative | 1E-174 | At1g55850 | 2.07 |
| 291 | 292 | putative protein | 0 | At5g46410 | 1.54 |
| 293 | 294 | putative polynucleotide phosphorylase | 1E-136 | At3g03710 | 1.53 |
| 295 | 296 | hypothetical protein | 0 | At1g19180 | 1.32 |
| 297 | 298 | hypothetical protein | 0 | At3g12270 | 1.83 |
| 299 | 300 | sugar transporter like protein | 0 | At4g36670 | 2.27 |
| 301 | 302 | hypothetical protein | 1E-105 | At2g39910 | 1.3 |
| 303 | 304 | putative phytochrome A | 0 | At1g09570 | 2.45 |
| 305 | 306 | hypothetical protein | 0 | At1g64600 | 1.49 |
| 307 | 308 | putative protein | 0 | At5g23610 | 1.6 |
| 309 | 310 | putative protein | 1E-177 | At3g56360 | 1.39 |
| 311 | 312 | cyclophylin -like protein | 0 | At3g63400 | 1.33 |
| 313 | 314 | unknown protein | 0 | At2g37940 | 1.35 |
| 315 | 316 | zinc finger protein, putative | 1E-53 | At1g75540 | 1.46 |
| 317 | 318 | putative protein kinase | 1E+00 | At2g24360 | 1.48 |
| 319 | 320 | putative glucosyltransferase | 0 | At2g15490 | 2.15 |
| 321 | 322 | unknown protein | 0 | At1g60140 | 1.72 |
| 323 | 324 | unknown protein | 0 | At1g43850 | 1.45 |
| 325 | 326 | hypothetical protein | 0 | At3g14120 | 1.77 |
| 327 | 328 | putative AP2 domain transcription factor | 0 | At2g41710 | 1.65 |
| 329 | 330 | transcriptional regulator protein, putative | 6E-71 | At3g26640 | 1.51 |
| 331 | 332 | hypothetical protein | 3E-02 | At1g55370 | 1.35 |

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|---|---|---|---|---|---|
| 333 | 334 | unknown protein | 0 | At3g28920 | 1.93 |
| 335 | 336 | hypothetical protein | 0 | At3g03750 | 1.43 |
| 337 | 338 | hypothetical protein | 2E+00 | At4g27610 | 1.34 |
| 339 | 340 | translation initiation factor eIF-2 beta chain - like protein | 2E+00 | At5g20920 | 1.33 |
| 341 | 342 | unknown protein | 0 | At2g26280 | 1.53 |
| 343 | 344 | unknown protein | 0 | At1g78420 | 1.39 |
| 345 | 346 | elongation factor, putative | 3E+00 | At1g56070 | 1.99 |
| 347 | 348 | anthranilate N-benzoyltransferase - like protein | 1E-120 | At5g01210 | 1.66 |
| 349 | 350 | putative protein | 1E-178 | At4g39680 | 1.43 |
| 351 | 352 | unknown protein | 0 | At3g05380 | 1.92 |
| 353 | 354 | splicing factor At-SRp40 | 0 | At4g25500 | 1.52 |
| 355 | 356 | cdc2-like protein kinase | 0 | At5g10270 | 1.77 |
| 357 | 358 | calcium-dependent protein kinase | 1E-169 | At3g57530 | 1.39 |
| 359 | 360 | phosphoprotein phosphatase, type 1 catalytic subunit | 0 | At2g29400 | 1.48 |
| 361 | 362 | putative mitochondrial translation elongation factor G | 0 | At2g45030 | 1.65 |
| 363 | 364 | long-chain-fatty-acid--CoA ligase-like protein | 0 | At5g27600 | 1.34 |
| 365 | 366 | cytochrome c, putative | 4E-26 | At3g27240 | 1.36 |
| 367 | 368 | En/Spm-like transposon protein | 0 | At2g40070 | 1.41 |
| 369 | 370 | putative phospho-ser/thr phosphatase | 0 | At4g03080 | 1.41 |
| 371 | 372 | chloroplast 50S ribosomal protein L22, putative | 6E-77 | At1g52370 | 1.4 |
| 373 | 374 | unknown protein | 0 | At2g15890 | 1.34 |
| 375 | 376 | putative protein | 0 | At4g26750 | 1.55 |
| 377 | 378 | receptor-protein kinase-like protein | 0 | At5g54380 | 2.59 |
| 379 | 380 | phosphoglycerate kinase, putative | 1E-155 | At3g12780 | 1.88 |
| 381 | 382 | putative HMG protein | 0 | At2g17560 | 1.45 |
| 383 | 384 | hypothetical protein | 0 | At1g76100 | 1.36 |
| 385 | 386 | protein kinase cdc2 homolog B | 0 | At3g54180 | 2.39 |
| 387 | 388 | T-complex protein 1, beta subunit | 0 | At5g20890 | 1.39 |
| 389 | 390 | proline oxidase, mitochondrial precursor (osmotic stress-induced) | 0 | At3g30775 | 1.45 |
| 391 | 392 | linker histone protein, putative | 1E-126 | At1g14900 | 1.33 |
| 393 | 394 | hypothetical protein | 0 | At1g27500 | 2.21 |
| 395 | 396 | ARF1-binding protein | 0 | At5g62010 | 1.58 |
| 397 | 398 | putative protein | 0 | At5g16270 | 1.37 |
| 399 | 400 | putative protein | 1E-173 | At5g13850 | 1.32 |
| 401 | 402 | src-2 like protein | 0 | At1g09070 | 2.19 |
| 403 | 404 | RAN2 small Ras-like GTP-binding nuclear protein (Ran-2) | 0 | At5g20020 | 1.3 |
| 405 | 406 | phosphoprotein phosphatase (PPX-1) | 0 | At4g26720 | 1.42 |
| 407 | 408 | nuclear protein-like | 0 | At5g64270 | 1.45 |
| 409 | 410 | ornithine carbamoyltransferase precursor | 0 | At1g75330 | 1.41 |
| 411 | 412 | unknown protein | 0 | At2g41650 | 1.67 |
| 413 | 414 | putative protein | 0 | At5g17640 | 1.66 |
| 415 | 416 | hypothetical protein | 0 | At1g57990 | 2.02 |
| 417 | 418 | hypothetical protein | 0 | At4g15760 | 1.64 |
| 419 | 420 | glycine-rich protein 2 (GRP2) | 0 | At4g38680 | 1.72 |
| 421 | 422 | hypothetical protein | 1E-113 | At2g41780 | 2.6 |
| 423 | 424 | RNA-binding protein, putative | 8E-95 | At3g20250 | 1.46 |
| 425 | 426 | gda-1, putative | 2E+00 | At3g27090 | 1.46 |
| 427 | 428 | beta-fructofuranosidase 1 | 0 | At3g13790 | 1.32 |
| 429 | 430 | 26S proteasome subunit 4-like protein | 0 | At4g29040 | 1.51 |
| 431 | 432 | putative protein | 1E-59 | At1g33980 | 1.42 |
| 433 | 434 | hypothetical protein | 0 | At1g57680 | 2.66 |
| 435 | 436 | unknown protein | 0 | At1g29980 | 1.98 |

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|---|---|---|---|---|---|
| 437 | 438 | 60S ribosomal protein - like | 0 | At5g02870 | 1.39 |
| 439 | 440 | REVOLUTA or interfascicular fiberless 1 | 0 | At5g60690 | 1.34 |
| 441 | 442 | RAC-like GTP-binding protein ARAC4 | 1E-180 | At1g20090 | 1.78 |
| 443 | 444 | unknown protein | 2E-42 | At3g07390 | 1.34 |
| 445 | 446 | unknown protein | 0 | At5g65660 | 1.7 |
| 447 | 448 | unknown protein | 1E-154 | At3g05040 | 1.52 |
| 449 | 450 | putative DNA gyrase subunit A | 1E-153 | At3g10690 | 2.2 |
| 451 | 452 | putative protein | 0 | At3g49170 | 1.53 |
| 453 | 454 | eukaryotic cap-binding protein (gb\|AAC17220.1) | 0 | At5g18110 | 1.41 |
| 455 | 456 | phosphoethanolamine N-methyltransferase, putative | 0 | At1g73600 | 1.62 |
| 457 | 458 | unknown protein | 0 | At2g30590 | 2.78 |
| 459 | 460 | RAN1 small Ras-like GTP-binding nuclear protein (Ran-1) | 0 | At5g20010 | 1.46 |
| 461 | 462 | putative protein | 1E-104 | At4g24290 | 1.32 |
| 463 | 464 | putative auxin-regulated protein | 0 | At2g45210 | 1.33 |
| 465 | 466 | adenylosuccinate synthetase | 0 | At3g57610 | 1.39 |
| 467 | 468 | putative protein | 0 | At5g14530 | 2.7 |
| 469 | 470 | putative ubiquitin activating enzyme E1 (ECR1) | 0 | At5g19180 | 1.63 |
| 471 | 472 | putative mitochondrial processing peptidase | 0 | At3g02090 | 1.4 |
| 473 | 474 | putative protein | 0 | At3g48530 | 1.55 |
| 475 | 476 | hypothetical protein | 0 | At1g20330 | 1.47 |
| 477 | 478 | hypothetical protein | 0 | At4g02590 | 1.36 |
| 479 | 480 | putative pyrophosphate-fructose-6-phosphate 1-phosphotransferase | 0 | At1g12000 | 1.49 |
| 481 | 482 | putative receptor-like protein kinase | 0 | At2g02220 | 1.55 |
| 483 | 484 | putative protein | 1E-104 | At4g02440 | 1.4 |
| 485 | 486 | non-phototropic hypocotyl, putative | 0 | At1g30440 | 1.57 |
| 487 | 488 | histone deacetylase | 0 | At5g63110 | 1.36 |
| 489 | 490 | putative protein | 0 | At5g66580 | 3.18 |
| 491 | 492 | multicatalytic endopeptidase complex, proteasome precursor, beta | 0 | At4g31300 | 1.42 |
| 493 | 494 | fibrillarin - like protein | 6E-77 | At4g25630 | 1.3 |
| 495 | 496 | hypothetical protein | 8E-45 | At1g54060 | 1.36 |
| 497 | 498 | histone H1, partial | 0 | At2g30620 | 1.58 |
| 499 | 500 | hypothetical protein | 0 | At3g09030 | 1.45 |
| 501 | 502 | enoyl-CoA hydratase - like protein | 0 | At4g31810 | 1.31 |
| 503 | 504 | unknown protein | 7E+00 | At2g27080 | 1.51 |
| 505 | 506 | myb-related transcription factor, putative | 0 | At3g23250 | 1.49 |
| 507 | 508 | Alcohol Dehydrogenase | 0 | At1g77120 | 5.09 |
| 509 | 510 | hypothetical protein | 1E-132 | At1g27590 | 1.38 |
| 511 | 512 | unknown protein | 0 | At1g14710 | 1.36 |
| 513 | 514 | putative receptor-like protein kinase | 0 | At2g13790 | 1.68 |
| 515 | 516 | putative protein | 0 | At5g14550 | 1.39 |
| 517 | 518 | homeobox protein knotted-1 like 4 (KNAT4) | 1E-165 | At5g11060 | 1.4 |
| 519 | 520 | putative protein | 1E-142 | At5g15540 | 1.47 |
| 521 | 522 | carbonyl reductase-like protein | 7E+00 | At5g51030 | 2.17 |
| 523 | 524 | hypothetical protein | 1E-50 | At1g53900 | 1.36 |
| 525 | 526 | aspartate--tRNA ligase - like protein | 0 | At4g31180 | 1.62 |
| 527 | 528 | unknown protein | 1E-121 | At3g06150 | 1.74 |
| 529 | 530 | amino acid transporter protein-like | 0 | At5g65990 | 1.59 |
| 531 | 532 | 12-oxophytodienoate reductase (OPR1) | 0 | At1g76680 | 1.43 |
| 533 | 534 | calnexin homolog | 6E-25 | At5g07340 | 1.39 |
| 535 | 536 | unknown protein | 0 | At1g61100 | 2.06 |

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|---|---|---|---|---|---|
| 537 | 538 | homogentisate 1,2-dioxygenase | 1E-78 | At5g54080 | 2.01 |
| 539 | 540 | glucosyltransferase -like protein | 0 | At4g34131 | 1.33 |
| 541 | 542 | putative protein | 4E-01 | At5g54890 | 1.35 |
| 543 | 544 | hypothetical protein | 0 | At1g76070 | 1.31 |
| 545 | 546 | putative protein | 1E-179 | At5g18310 | 1.56 |
| 547 | 548 | DNA binding protein ACBF - like | 0 | At5g19350 | 1.36 |
| 549 | 550 | hypothetical protein | 0 | At1g17210 | 1.69 |
| 551 | 552 | putative protein | 1E-111 | At5g51220 | 1.46 |
| 553 | 554 | RING finger protein | 0 | At3g61460 | 2.14 |
| 555 | 556 | putative protein | 0 | At5g18580 | 1.32 |
| 557 | 558 | putative protein kinase | 0 | At2g31010 | 1.35 |
| 559 | 560 | chloroplast nucleoid DNA binding protein, putative | 0 | At1g01300 | 1.49 |
| 561 | 562 | unknown protein | 1E-143 | At1g31130 | 1.4 |
| 563 | 564 | splicing factor, putative | 1E+00 | At1g14650 | 1.56 |
| 565 | 566 | putative TCP3 gb\|AAC24010. | 0 | At1g53230 | 1.38 |
| 567 | 568 | unknown protein | 0 | At1g72790 | 1.71 |
| 569 | 570 | ribosomal protein S6 - like | 0 | At4g31700 | 1.38 |
| 571 | 572 | auxin-resistance protein AXR1 | 0 | At1g05180 | 1.36 |
| 573 | 574 | putative protein | 0 | At5g11030 | 1.43 |
| 575 | 576 | putative 60S acidic ribosomal protein P0 | 0 | At3g09200 | 1.47 |
| 577 | 578 | mismatch binding protein, putative | 0 | At3g24320 | 2.1 |
| 579 | 580 | T-complex chaperonin protein , epsilon subunit | 0 | At1g24510 | 1.47 |
| 581 | 582 | putative protein | 0 | At4g24120 | 1.56 |
| 583 | 584 | putative protein | 4E-38 | At5g53900 | 1.79 |
| 585 | 586 | histidine transport protein (PTR2-B) | 0 | At2g02040 | 1.37 |
| 587 | 588 | unknown protein | 0 | At3g10490 | 1.43 |
| 589 | 590 | tubulin alpha-5 chain-like protein | 0 | At5g19770 | 1.6 |
| 591 | 592 | putative non-LTR retroelement reverse transcriptase | 6E+00 | At2g15510 | 4.71 |
| 593 | 594 | unknown protein | 1E-179 | At2g41010 | 1.33 |
| 595 | 596 | putative chloroplast outer envelope 86-like protein | 0 | At4g02510 | 1.45 |
| 597 | 598 | serine/threonine-specific protein kinase NAK | 0 | At5g02290 | 1.56 |
| 599 | 600 | unknown protein | 0 | At2g34680 | 1.45 |
| 601 | 602 | hypothetical protein | 0 | At1g43170 | 1.69 |
| 603 | 604 | phospholipase D, putative, 5' partial | 0 | At3g16785 | 1.5 |
| 605 | 606 | CTP synthase-like protein | 0 | At1g30820 | 1.58 |
| 607 | 608 | nitrilase 2 | 0 | At3g44300 | 1.84 |
| 609 | 610 | putative mitogen activated protein kinase kinase | 0 | At3g04910 | 1.34 |
| 611 | 612 | putative protein | 0 | At4g27450 | 1.4 |
| 613 | 614 | Phospholipase like protein | 0 | At4g38550 | 1.9 |
| 615 | 616 | endomembrane-associated protein | 3E-41 | At4g20260 | 1.83 |
| 617 | 618 | leucine-rich receptor-like protein kinase, putative | 0 | At1g72180 | 2.13 |
| 619 | 620 | putative protein | 8E-01 | At4g25930 | 1.54 |
| 621 | 622 | WD-40 repeat protein MSI1 (sp\|O22467) | 0 | At5g58230 | 1.72 |
| 623 | 624 | oxysterol-binding protein - like | 1E-171 | At5g59420 | 1.31 |
| 625 | 626 | putative protein | 1E-178 | At4g21840 | 1.4 |
| 627 | 628 | blue copper binding protein | 1E-50 | At5g20230 | 2.3 |
| 629 | 630 | UV-damaged DNA-binding protein- like | 6 E-9 | At4g21100 | 1.46 |
| 631 | 632 | fatty acid hydroxylase (FAH1) | 0 | At2g34770 | 1.96 |
| 633 | 634 | putative thymidine kinase | 0 | At3g07800 | 8.44 |
| 635 | 636 | hypothetical protein | 0 | At1g79380 | 1.41 |
| 637 | 638 | unknown protein | 0 | At2g15860 | 1.36 |
| 639 | 640 | flower pigmentation protein ATAN11 | 0 | At1g12910 | 1.41 |

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|---|---|---|---|---|---|
| 641 | 642 | hypothetical protein | 0 | At1g56290 | 1.33 |
| 643 | 644 | putative protein | 0 | At3g62630 | 1.38 |
| 645 | 646 | SNF-2 like RING finger | 0 | At1g61140 | 1.42 |
| 647 | 648 | unknown protein | 0 | At3g16310 | 1.49 |
| 649 | 650 | putative glucosyl transferase | 0 | At2g36800 | 1.36 |
| 651 | 652 | putative protein | 0 | At4g25170 | 1.92 |
| 653 | 654 | hypothetical protein | 9E-39 | At4g00450 | 1.36 |
| 655 | 656 | glutathione S-transferase | 0 | At2g30860 | 1.49 |
| 657 | 658 | unknown protein, 3' partial | 0 | At3g15095 | 1.42 |
| 659 | 660 | unknown protein | 0 | At3g21080 | 1.31 |
| 661 | 662 | TCH4 protein (gb\|AAA92363.1) | 0 | At5g57560 | 1.92 |
| 663 | 664 | putative protein | 0 | At3g61600 | 1.34 |
| 665 | 666 | receptor-like kinase, putative | 0 | At3g23750 | 2.06 |
| 667 | 668 | putative 2,3-bisphosphoglycerate-independent phosphoglycerate | 0 | At1g09780 | 1.34 |
| 669 | 670 | putative protein | 0 | At5g14250 | 1.51 |
| 671 | 672 | DnaJ homologue (gb\|AAB91418.1\|) | 0 | At5g06910 | 2.32 |
| 673 | 674 | hypothetical protein | 0 | At1g33250 | 1.35 |
| 675 | 676 | unknown protein | 0 | At2g19800 | 1.81 |
| 677 | 678 | aspartate carbamoyltransferase precursor (aspartate | 3E-84 | At3g20330 | 1.49 |
| 679 | 680 | hypothetical protein | 0 | At1g16520 | 1.35 |
| 681 | 682 | unknown protein | 5E+00 | At1g48620 | 1.33 |
| 683 | 684 | putative protein | 1E+00 | At4g35750 | 1.39 |
| 685 | 686 | hypothetical protein | 1E-55 | At3g13620 | 1.79 |
| 687 | 688 | RNA helicase, DRH1 | 1E-179 | At3g01540 | 1.56 |
| 689 | 690 | putative 3-oxoacyl [acyl-carrier protein] reductase | 0 | At1g24360 | 1.42 |
| 691 | 692 | putative cellular apoptosis susceptibility protein | 1E-142 | At2g46520 | 1.43 |
| 693 | 694 | hypothetical protein | 0 | At1g01540 | 1.31 |
| 695 | 696 | starch branching enzyme II | 2E-61 | At2g36390 | 1.36 |
| 697 | 698 | 40S ribosomal protein - like | 0 | At5g15200 | 1.32 |
| 699 | 700 | putative protein | 0 | At4g13640 | 1.33 |
| 701 | 702 | putative protein | 0 | At3g45970 | 3.22 |
| 703 | 704 | hypothetical protein | 0 | At1g66160 | 1.31 |
| 705 | 706 | AP2 domain containing protein RAP2.3 | 2 E-9 | At3g16770 | 1.51 |
| 707 | 708 | putative protein | 1E-47 | At5g02880 | 1.32 |
| 709 | 710 | NADH-dependent glutamate synthase | 0 | At5g53460 | 2.25 |
| 711 | 712 | arginine/serine rich splicing factor RSP3 | 4E-59 | At3g61860 | 1.31 |
| 713 | 714 | hypothetical protein | 1E-134 | At1g55880 | 1.37 |
| 715 | 716 | translation initiation factor eIF3 - like protein | 6E-77 | At4g20980 | 1.45 |
| 717 | 718 | putative serine/threonine protein phosphatase catalytic subunit, | 0 | At2g42500 | 1.38 |
| 719 | 720 | unknown protein | 1E-105 | At1g33480 | 1.91 |
| 721 | 722 | COP1-interacting protein CIP8 | 0 | At5g64920 | 1.4 |
| 723 | 724 | nonphototropic hypocotyl 1 | 6E+00 | At3g45780 | 1.47 |
| 725 | 726 | putative protein | 1E-78 | At5g10860 | 1.32 |
| 727 | 728 | putative protein | 0 | At5g19750 | 1.37 |
| 729 | 730 | putative protein | 1E-127 | At3g52500 | 1.39 |
| 731 | 732 | putative protein | 0 | At4g10280 | 1.76 |
| 733 | 734 | cytochrome P450 monooxygenase | 0 | At4g31500 | 1.35 |
| 735 | 736 | ethylene responsive element binding factor | 1E-104 | At4g17500 | 1.33 |
| 737 | 738 | hypothetical protein | 0 | At1g17620 | 1.37 |
| 739 | 740 | unknown protein | 1E-123 | At3g07390 | 1.42 |
| 741 | 742 | putative protein kinase | 0 | At3g02880 | 1.46 |
| 743 | 744 | DNA repair protein RAD23 homolog | 0 | At5g38470 | 1.42 |

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|---|---|---|---|---|---|
| 745 | 746 | GTP-binding protein - like | 1E-25 | At5g03520 | 1.57 |
| 747 | 748 | putative protein | 0 | At3g63500 | 1.4 |
| 749 | 750 | putative adenylate kinase | 4E+00 | At2g39270 | 1.37 |
| 751 | 752 | protein kinase - like | 6E-46 | At5g59010 | 1.42 |
| 753 | 754 | unknown protein | 0 | At3g04630 | 1.58 |
| 755 | 756 | RNA binding protein | 0 | At1g73490 | 1.32 |
| 757 | 758 | putative phospholipase D | 0 | At3g15730 | 1.51 |
| 759 | 760 | importin alpha | 1E-115 | At3g06720 | 1.45 |
| 761 | 762 | RING-H2 finger protein RHF2a | 0 | At5g22000 | 1.43 |
| 763 | 764 | putative protein | 2E-93 | At4g19160 | 1.3 |
| 765 | 766 | putative protein | 0 | At4g32440 | 1.41 |
| 767 | 768 | putative protein phosphatase type 2C | 0 | At3g15260 | 1.61 |
| 769 | 770 | putative protein | 0 | At5g39890 | 1.31 |
| 771 | 772 | ribosomal protein | 0 | At4g16720 | 1.42 |
| 773 | 774 | dormancy-associated protein | 9E+00 | At1g28330 | 2.01 |
| 775 | 776 | auxin-inducible gene (IAA2) | 0 | At3g23030 | 1.65 |
| 777 | 778 | unknown protein | 5E+00 | At1g76010 | 1.54 |
| 779 | 780 | protein kinase ADK1-like protein | 1E+00 | At4g28540 | 1.96 |
| 781 | 782 | putative protein | 0 | At4g24210 | 1.36 |
| 783 | 784 | hypothetical protein | 0 | At1g79530 | 1.4 |
| 785 | 786 | putative trehalose-6-phosphate synthase | 0 | At1g68020 | 1.45 |
| 787 | 788 | adenylate kinase | 0 | At5g63400 | 1.39 |
| 789 | 790 | putative proline-rich protein precursor | 0 | At1g73840 | 1.56 |
| 791 | 792 | putative protein | 5E-87 | At5g14370 | 1.37 |
| 793 | 794 | hypothetical protein | 0 | At4g33290 | 1.7 |
| 795 | 796 | cytochrome P450 monooxygenase (CYP71B3) | 0 | At3g26220 | 1.32 |
| 797 | 798 | TMV resistance protein N - like | 0 | At4g19530 | 1.5 |
| 799 | 800 | hypothetical protein | 9E-70 | At1g54830 | 1.33 |
| 801 | 802 | 3-ketoacyl-CoA thiolase | 0 | At2g33150 | 1.87 |
| 803 | 804 | putative protein | 0 | At3g54350 | 1.35 |
| 805 | 806 | hypothetical protein | 1E-170 | At4g02680 | 1.36 |
| 807 | 808 | putative bHLH transcription factor | 0 | At2g46510 | 1.35 |
| 809 | 810 | RNA-binding protein, putative | 5E-84 | At3g26420 | 1.55 |
| 811 | 812 | putative lectin | 3E-20 | At3g09190 | 1.67 |
| 813 | 814 | xyloglucan endotransglycosylase, putative | 0 | At3g23730 | 2.85 |
| 815 | 816 | unknown protein | 2E-33 | At2g41170 | 1.32 |
| 817 | 818 | putative protein | 3E-78 | At3g57150 | 1.67 |
| 819 | 820 | putative glucose regulated repressor protein | 0 | At2g25490 | 1.81 |
| 821 | 822 | putative AP2 domain containing protein RAP2.4 gi\|2281633 | 1E-150 | At1g78080 | 1.82 |
| 823 | 824 | putative sulfate transporter | 0 | At1g80310 | 1.51 |
| 825 | 826 | G protein alpha subunit 1 (GPA1) | 0 | At2g26300 | 1.44 |
| 827 | 828 | protochlorophyllide reductase precursor | 0 | At4g27440 | 2.39 |
| 829 | 830 | Shaggy related protein kinase tetha | 0 | At4g00720 | 1.52 |
| 831 | 832 | putative protein kinase | 0 | At3g01300 | 1.49 |
| 833 | 834 | RNA-binding protein-like protein | 0 | At3g47160 | 1.31 |
| 835 | 836 | unknown protein | 1E-150 | At5g24670 | 1.47 |
| 837 | 838 | zinc finger protein ZFP8 | 1E-144 | At2g41940 | 1.42 |
| 839 | 840 | GTP binding protein beta subunit | 0 | At4g34460 | 1.54 |
| 841 | 842 | copia-like retroelement pol polyprotein | 0 | At2g22680 | 1.4 |
| 843 | 844 | CONSTANS-like B-box zinc finger protein-like | 0 | At5g57660 | 1.36 |
| 845 | 846 | unknown protein | 3E-71 | At3g10640 | 1.33 |
| 847 | 848 | putative protein | 0 | At4g24690 | 1.91 |

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|---|---|---|---|---|---|
| 849 | 850 | NADH dehydrogenase | 1E-124 | At5g08530 | 1.42 |
| 851 | 852 | unknown protein | 0 | At1g73820 | 1.35 |
| 853 | 854 | monosaccharide transport protein, STP4 | 8 E-9 | At3g19930 | 1.58 |
| 855 | 856 | globulin-like protein | 0 | At1g07750 | 1.61 |
| 857 | 858 | putative transitional endoplasmic reticulum ATPase | 2E-58 | At3g09840 | 1.51 |
| 859 | 860 | putative monodehydroascorbate reductase | 0 | At1g63940 | 1.39 |
| 861 | 862 | anthranilate phosphoribosyltransferase-like protein | 0 | At3g57880 | 1.42 |
| 863 | 864 | H+-transporting ATP synthase chain 9 - like protein | 6E-25 | At4g32260 | 1.83 |
| 865 | 866 | hypothetical protein | 0 | At1g02810 | 2.31 |
| 867 | 868 | calmodulin-like protein | 3E-63 | At2g41410 | 1.52 |
| 869 | 870 | putative protein | 0 | At5g15350 | 2.75 |
| 871 | 872 | glutathione S-transferase | 0 | At2g30870 | 1.54 |
| 873 | 874 | putative SWI/SNF complex subunit SW13 | 1E-138 | At2g33610 | 1.32 |
| 875 | 876 | MAP kinase kinase 2 | 0 | At4g29810 | 1.39 |
| 877 | 878 | adenosylhomocysteinase | 1E-134 | At4g13940 | 2.07 |
| 879 | 880 | putative protein | 0 | At5g27760 | 1.4 |
| 881 | 882 | unknown protein | 0 | At2g47450 | 1.67 |
| 883 | 884 | putative protein | 0 | At4g33050 | 2.2 |
| 885 | 886 | 50S ribosomal protein L12-C | 1E-138 | At3g27850 | 1.38 |
| 887 | 888 | 26S proteasome AAA-ATPase subunit RPT4a (gb\|AAF22524.1) | 0 | At5g43010 | 1.4 |
| 889 | 890 | unknown protein | 8E-01 | At3g01690 | 1.31 |
| 891 | 892 | lipid transfer protein; glossy1 homolog | 0 | At5g57800 | 1.39 |
| 893 | 894 | indoleacetic acid (IAA)-inducible gene (IAA7) | 1 E-7 | At3g23050 | 1.52 |
| 895 | 896 | histone H2B - like protein | 0 | At5g59910 | 2.16 |
| 897 | 898 | putative RNA helicase | 0 | At3g06480 | 1.47 |
| 899 | 900 | unknown protein | 8E-64 | At1g19310 | 1.44 |
| 901 | 902 | unknown protein | 4E-96 | At2g18440 | 1.38 |
| 903 | 904 | unknown protein | 0 | At1g68220 | 1.59 |
| 905 | 906 | unknown protein | 1E-142 | At2g20570 | 1.35 |
| 907 | 908 | putative replication factor | 1E-124 | At1g21690 | 3.3 |
| 909 | 910 | U2 snRNP auxiliary factor, small subunit | 0 | At5g42820 | 1.55 |
| 911 | 912 | replication factor C - like | 0 | At5g27740 | 1.45 |
| 913 | 914 | nuclear receptor binding factor-like protein | 0 | At3g45770 | 1.62 |
| 915 | 916 | putative glycosyl transferase | 0 | At1g24170 | 2.39 |
| 917 | 918 | histone H2A-like protein | 4E-53 | At5g27670 | 1.62 |
| 919 | 920 | putative protein | 1E-125 | At5g48960 | 1.43 |
| 921 | 922 | hypothetical protein | 0 | At1g53740 | 1.42 |
| 923 | 924 | splicing factor - like protein | 0 | At3g53500 | 1.39 |
| 925 | 926 | unknown protein | 0 | At1g50510 | 1.32 |
| 927 | 928 | Fe(II) transport protein | 0 | At4g19690 | 1.37 |
| 929 | 930 | hypothetical protein | 0 | At1g61730 | 1.43 |
| 931 | 932 | unknown protein | 7 E-9 | At2g47440 | 2.5 |
| 933 | 934 | cold-regulated protein COR6.6 (KIN2) | 0 | At5g15970 | 3.03 |
| 935 | 936 | putative cytochrome C | 0 | At1g22840 | 1.3 |
| 937 | 938 | unknown protein | 0 | At1g68580 | 2.13 |
| 939 | 940 | putative Ser/Thr protein kinase | 0 | At1g16270 | 1.37 |
| 941 | 942 | pseudogene | 1E-138 | At2g25970 | 2.15 |
| 943 | 944 | unknown protein | 0 | At3g06380 | 1.67 |
| 945 | 946 | Tic22, putative | 3E-84 | At3g23710 | 2.14 |
| 947 | 948 | unknown protein | 0 | At1g09250 | 1.55 |
| 949 | 950 | hypothetical protein | 0 | At1g72930 | 1.91 |
| 951 | 952 | hypothetical protein | 2E+00 | At1g68820 | 1.43 |

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|---|---|---|---|---|---|
| 953 | 954 | histone H1 | 0 | At2g18050 | 1.75 |
| 955 | 956 | unknown protein | 0 | At1g08630 | 1.45 |
| 957 | 958 | unknown protein, 5'partial | 0 | At3g18035 | 3.31 |
| 959 | 960 | unknown protein | 0 | At1g04140 | 1.37 |
| 961 | 962 | HAL3A protein | 0 | At3g18030 | 1.43 |
| 963 | 964 | phi-1-like protein | 0 | At5g64260 | 3.38 |
| 965 | 966 | hypothetical protein | 0 | At1g12770 | 1.35 |
| 967 | 968 | pollen specific protein SF21 | 0 | At5g56750 | 1.45 |
| 969 | 970 | cysteine proteinase inhibitor like protein | 1E-159 | At4g16500 | 1.33 |
| 971 | 972 | 20S proteasome subunit C8 (PAG1/PRC8 ARATH) | 1E-130 | At2g27020 | 1.36 |
| 973 | 974 | nodulin-like protein | 1E-99 | At1g75500 | 1.34 |
| 975 | 976 | hypothetical protein | 0 | At1g72900 | 2.04 |
| 977 | 978 | hypothetical protein | 0 | At2g35230 | 1.42 |
| 979 | 980 | arm repeat containing protein homolog | 0 | At3g46510 | 1.4 |
| 981 | 982 | putative protein | 0 | At5g67480 | 1.76 |
| 983 | 984 | putative leucyl-tRNA synthetase | 1E-118 | At1g09620 | 1.52 |
| 985 | 986 | Putative UDP-glucose glucosyltransferase | 1E-164 | At1g22400 | 2.34 |
| 987 | 988 | alanine aminotransferase, putative | 0 | At1g17290 | 1.66 |
| 989 | 990 | 26S proteasome AAA-ATPase subunit RPT6a | 0 | At5g19990 | 1.36 |
| 991 | 992 | Ruv DNA-helicase-like protein | 0 | At5g22330 | 1.59 |
| 993 | 994 | small nuclear ribonucleoprotein, putative | 0 | At1g65700 | 1.33 |
| 995 | 996 | unknown protein | 0 | At2g38310 | 2.79 |
| 997 | 998 | protein phosphatase type 1 PP1BG | 3E-91 | At4g11240 | 1.51 |
| 999 | 1000 | hypothetical protein | 3E-41 | At2g43410 | 2.1 |
| 1001 | 1002 | putative protein | 0 | At5g58600 | 1.42 |
| 1003 | 1004 | nodulin-like protein | 0 | At1g80530 | 2.07 |
| 1005 | 1006 | putative protein | 0 | At5g56170 | 1.65 |
| 1007 | 1008 | dihydroxyacetone kinase, putative | 1E-167 | At3g17770 | 1.67 |
| 1009 | 1010 | ribsomal protein - like | 1E-155 | At5g09770 | 1.44 |
| 1011 | 1012 | 101 kDa heat shock protein; HSP101-like protein | 0 | At5g57710 | 1.34 |
| 1013 | 1014 | unknown protein | 0 | At5g51340 | 1.48 |
| 1015 | 1016 | unknown protein | 0 | At3g05730 | 1.46 |
| 1017 | 1018 | putative protein | 2E+00 | At5g67570 | 2.6 |
| 1019 | 1020 | mitochondrial chaperonin (HSP60) | 0 | At2g33210 | 1.75 |
| 1021 | 1022 | putative protein | 1E-177 | At3g63270 | 1.34 |
| 1023 | 1024 | growth factor like protein | 0 | At4g12720 | 1.78 |
| 1025 | 1026 | RNA helicase, putative | 0 | At3g19760 | 1.54 |
| 1027 | 1028 | pseudogene | 1E-142 | At2g34760 | 1.81 |
| 1029 | 1030 | hypothetical protein | 0 | At3g21740 | 1.52 |
| 1031 | 1032 | shaggy-like kinase beta | 0 | At3g61160 | 1.36 |
| 1033 | 1034 | unknown protein | 1E-165 | At1g20100 | 1.35 |
| 1035 | 1036 | 24-sterol C-methyltransferase | 1E-143 | At5g13710 | 1.41 |
| 1037 | 1038 | WD-40 repeat protein (MSI3) | 0 | At4g35050 | 4.89 |
| 1039 | 1040 | hypothetical protein | 0 | At1g67120 | 1.51 |
| 1041 | 1042 | putative protein (fragment) | 0 | At5g14930 | 1.46 |
| 1043 | 1044 | putative protein | 1 E-6 | At5g54180 | 1.78 |
| 1045 | 1046 | hypothetical protein | 1E-126 | At1g20570 | 1.43 |
| 1047 | 1048 | calcium-dependent protein kinase | 0 | At5g66210 | 2.96 |
| 1049 | 1050 | nitrilase 2 | 1E-127 | At3g44300 | 1.88 |
| 1051 | 1052 | methionyl-tRNA synthetase - like protein | 1E-173 | At4g13780 | 1.33 |
| 1053 | 1054 | putative protein | 0 | At4g24230 | 1.58 |
| 1055 | 1056 | putative protein | 2E-76 | At5g19330 | 1.33 |

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|---|---|---|---|---|---|
| 1057 | 1058 | caffeoyl-CoA O-methyltransferase - like protein | 1E-166 | At4g34050 | 1.41 |
| 1059 | 1060 | putative DNA binding protein | 0 | At4g27000 | 1.43 |
| 1061 | 1062 | unknown protein | 0 | At1g55270 | 1.4 |
| 1063 | 1064 | carbamoyl phosphate synthetase large chain (carB) | 0 | At1g29900 | 1.5 |
| 1065 | 1066 | hypothetical protein | 6E+00 | At4g02680 | 2.73 |
| 1067 | 1068 | putative RNA helicase | 0 | At3g22310 | 1.53 |
| 1069 | 1070 | molybdopterin synthase sulphurylase (gb\|AAD18050.1) | 0 | At5g55130 | 1.77 |
| 1071 | 1072 | inner mitochondrial membrane protein, putative | 0 | At1g17530 | 1.55 |
| 1073 | 1074 | putative protein kinase | 0 | At3g08760 | 1.9 |
| 1075 | 1076 | putative JUN kinase activator protein | 0 | At1g22920 | 1.42 |
| 1077 | 1078 | thaumatin, putative | 0 | At1g75800 | 1.56 |
| 1079 | 1080 | DNA-binding protein | 0 | At3g14230 | 1.54 |
| 1081 | 1082 | unknown protein | 0 | At2g01710 | 1.34 |
| 1083 | 1084 | putative calcium binding protein | 0 | At2g43290 | 1.57 |
| 1085 | 1086 | class 1 non-symbiotic hemoglobin (AHB1) | 5E-93 | At2g16060 | 1.86 |
| 1087 | 1088 | glycine-rich RNA binding protein; putative | 2E-52 | At3g23830 | 1.38 |
| 1089 | 1090 | unknown protein | 2E-37 | At2g01190 | 1.3 |
| 1091 | 1092 | hydoxyethylthiazole kinase, putative | 2E-71 | At3g24030 | 1.35 |
| 1093 | 1094 | putative protein translocase | 0E+00 | At2g37410 | 1.51 |
| 1095 | 1096 | putative protein | 5E-02 | At5g61560 | 1.31 |
| 1097 | 1098 | hypothetical protein | 7E-02 | At1g35600 | 1.56 |
| 1099 | 1100 | ethylene-insensitive 3 | 0 | At3g20770 | 1.5 |
| 1101 | 1102 | lipoxygenase AtLOX2 | 0 | At3g45140 | 1.57 |
| 1103 | 1104 | putative phosphatidic acid phosphatase | 0 | At2g01180 | 1.85 |
| 1105 | 1106 | unknown protein | 5 E-5 | At1g80860 | 1.3 |
| 1107 | 1108 | unknown protein | 2 E-15 | At3g28180 | 1.64 |
| 1109 | 1110 | LOB domain protien 41 | 0 | At3g02550 | 4.01 |
| 1111 | 1112 | putative protein | 2E-02 | At5g22260 | 1.95 |
| 1113 | 1114 | actin - like protein | 1E-180 | At3g60830 | 1.36 |
| 1115 | 1116 | DEAD-box protein abstrakt | 0 | At5g51280 | 1.53 |
| 1117 | 1118 | putative DNA polymerase epsilon catalytic subunit | 2E+00 | At2g27120 | 2.87 |
| 1119 | 1120 | unknown protein | 6E-59 | At5g48020 | 1.4 |
| 1121 | 1122 | protein kinase C inhibitor-like protein | 0 | At3g56490 | 1.58 |
| 1123 | 1124 | putative PRP19-like spliceosomal protein | 0 | At2g33340 | 1.7 |
| 1125 | 1126 | germin-like protein | 0 | At1g72610 | 1.67 |
| 1127 | 1128 | putative protein | 1 E-5 | At5g10050 | 1.32 |
| 1129 | 1130 | putative protein | 0 | At4g34950 | 1.96 |
| 1131 | 1132 | zinc finger protein | 0 | At5g66730 | 1.37 |
| 1133 | 1134 | chaperonin gamma chain - like protein | 1E-176 | At5g26360 | 1.67 |
| 1135 | 1136 | WD-40 protein | 7E+00 | At4g07410 | 1.42 |
| 1137 | 1138 | putative DNA-binding protein | 0 | At4g12080 | 1.4 |
| 1139 | 1140 | beta-glucosidase, putative | 0 | At1g52400 | 1.66 |
| 1141 | 1142 | hypothetical protein | 1E-44 | At2g23140 | 1.66 |
| 1143 | 1144 | homeobox protein | 1E-43 | At3g61150 | 1.63 |
| 1145 | 1146 | glycine-rich protein | 0 | At4g36020 | 1.82 |
| 1147 | 1148 | unknown protein | 0 | At3g01460 | 1.37 |
| 1149 | 1150 | hypothetical protein | 1E-134 | At4g28190 | 1.4 |
| 1151 | 1152 | predicted protein | 5E-37 | At4g32010 | 1.34 |
| 1153 | 1154 | N-myristoyl transferase | 1E-157 | At5g57020 | 1.37 |
| 1155 | 1156 | putative protein | 0 | At4g36780 | 1.61 |
| 1157 | 1158 | unknown protein | 2E-01 | At5g48240 | 1.64 |
| 1159 | 1160 | unknown protein | 0 | At1g21630 | 1.55 |

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|---|---|---|---|---|---|
| 1161 | 1162 | unknown protein | 1E-102 | At1g07360 | 1.74 |
| 1163 | 1164 | lysyl-tRNA synthetase | 1E-96 | At3g11710 | 1.38 |
| 1165 | 1166 | unknown protein | 0 | At3g07780 | 1.51 |
| 1167 | 1168 | tryptophan synthase beta chain 1 precursor (sp\|P14671) | 1E-102 | At5g54810 | 1.55 |
| 1169 | 1170 | putative protein | 8E-98 | At4g25620 | 1.81 |
| 1171 | 1172 | RuvB DNA helicase-like protein | 0 | At5g67630 | 1.32 |
| 1173 | 1174 | putative pectin methylesterase | 0 | At3g14310 | 1.43 |
| 1175 | 1176 | putative cytidine deaminase | 0 | At2g19570 | 1.41 |
| 1177 | 1178 | hypothetical protein | 0 | At3g12400 | 1.42 |
| 1179 | 1180 | 1-aminocyclopropane-1-carboxylate synthase -like protein | 0 | At4g26200 | 1.54 |
| 1181 | 1182 | peroxidase | 3E-88 | At2g38380 | 2.11 |
| 1183 | 1184 | 2-oxoglutarate dehydrogenase, E1 component | 0 | At5g65750 | 1.44 |
| 1185 | 1186 | xylosidase | 0 | At5g49360 | 1.93 |
| 1187 | 1188 | ethylene responsive element binding factor 4 | 0 | At3g15210 | 1.7 |
| 1189 | 1190 | putative protein | 2E+00 | At5g46650 | 3.54 |
| 1191 | 1192 | eukaryotic protein synthesis initiation factor 4A | 0 | At3g13920 | 1.35 |
| 1193 | 1194 | Unknown protein | 0 | At1g76970 | 2.34 |
| 1195 | 1196 | hypothetical protein | 0 | At1g19380 | 1.54 |
| 1197 | 1198 | unknown protein | 0 | At5g49640 | 1.78 |
| 1199 | 1200 | putative xyloglucan-specific glucanase | 0 | At2g01850 | 1.58 |
| 1201 | 1202 | similar to nucellin gb\|AAB96882.1 | 1E-106 | At1g49050 | 1.5 |
| 1203 | 1204 | unknown protein | 0 | At3g29390 | 1.33 |
| 1205 | 1206 | putative protein | 0 | At3g62190 | 1.58 |
| 1207 | 1208 | putative malate dehydrogenase | 0 | At1g04410 | 1.34 |
| 1209 | 1210 | putative isocitrate lyase | 1E-153 | At3g21720 | 3.08 |
| 1211 | 1212 | DNA-binding protein | 1E-160 | At3g14230 | 1.48 |
| 1213 | 1214 | histone H4-like protein | 0 | At3g46320 | 2.55 |
| 1215 | 1216 | putative dehydrogenase | 0 | At1g71170 | 1.47 |
| 1217 | 1218 | alanine--tRNA ligase, putative | 0 | At1g50200 | 1.38 |
| 1219 | 1220 | oligopeptidase A - like protein | 1E-172 | At5g10540 | 1.43 |
| 1221 | 1222 | putative protein | 0 | At5g62620 | 1.32 |
| 1223 | 1224 | permease | 0 | At5g49990 | 1.3 |
| 1225 | 1226 | DEAD BOX RNA helicase RH15 | 1E-129 | At5g11200 | 1.4 |
| 1227 | 1228 | lipoamide dehydrogenase precursor | 1E-128 | At3g17240 | 1.38 |
| 1229 | 1230 | hypothetical protein | 0 | At1g15170 | 1.75 |
| 1231 | 1232 | xyloglucan endo-1,4-beta-D-glucanase (XTR-6) | 0 | At4g25810 | 1.95 |
| 1233 | 1234 | histone H2B like protein (emb\|CAA69025.1) | 7E-34 | At5g22880 | 1.91 |
| 1235 | 1236 | S-receptor kinase homolog 2 precursor | 1E+00 | At5g60900 | 2.61 |
| 1237 | 1238 | 60S ribosomal protein L2 | 7E-48 | At2g18020 | 1.58 |
| 1239 | 1240 | unknown protein | 0 | At1g23030 | 1.98 |
| 1241 | 1242 | zinc finger protein, putative | 0 | At1g34370 | 1.51 |
| 1243 | 1244 | putative protein | 3 E-8 | At4g05150 | 1.38 |
| 1245 | 1246 | aldose 1-epimerase - like protein | 5E-25 | At3g47800 | 1.88 |
| 1247 | 1248 | cinnamoyl-CoA reductase - like protein | 0 | At5g58490 | 1.35 |
| 1249 | 1250 | putative NADP-dependent glyceraldehyde-3-phosphate dehydrogenase | 0 | At2g24270 | 1.43 |
| 1251 | 1252 | isp4 like protein | 0 | At4g16370 | 1.77 |
| 1253 | 1254 | putative protein | 0 | At4g08350 | 1.32 |
| 1255 | 1256 | calmodulin-related protein 2, touch-induced (TCH2) | 0 | At5g37770 | 1.55 |
| 1257 | 1258 | 20S proteasome subunit PAD2 (gb\|AAC32059.1) | 0 | At5g66140 | 1.5 |
| 1259 | 1260 | glucosidase II alpha subunit | 0 | At5g63840 | 1.35 |
| 1261 | 1262 | putative GAR1 protein | 0 | At3g03920 | 1.74 |

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|---|---|---|---|---|---|
| 1263 | 1264 | putative protein | 3E-45 | At5g08450 | 1.79 |
| 1265 | 1266 | glutamate dehydrogenase (EC 1.4.1.-) 1 (pir\|\|S71217) | 0 | At5g18170 | 1.47 |
| 1267 | 1268 | putative protein | 0 | At5g06660 | 1.32 |
| 1269 | 1270 | Nonclathrin coat protein gamma - like protein | 1E-143 | At4g34450 | 1.43 |
| 1271 | 1272 | unknown protein | 0 | At3g17860 | 1.6 |
| 1273 | 1274 | similar to senescence-associated protein | 0 | At2g23810 | 1.59 |
| 1275 | 1276 | putative protein | 0 | At5g60420 | 1.31 |
| 1277 | 1278 | unknown protein | 0 | At1g28260 | 1.36 |
| 1279 | 1280 | shaggy-like protein kinase etha (EC 2.7.1.-) | 0 | At4g18710 | 1.37 |
| 1281 | 1282 | putative 26S protease regulatory subunit 6A | 0 | At1g09100 | 1.47 |
| 1283 | 1284 | unknown protein | 0 | At3g21140 | 1.49 |
| 1285 | 1286 | dynamin-like protein | 0 | At2g14120 | 1.4 |
| 1287 | 1288 | scarecrow-like 1 | 2E-47 | At1g21450 | 1.75 |
| 1289 | 1290 | unknown protein | 7E-40 | At3g02710 | 1.3 |
| 1291 | 1292 | putative protein | 0 | At5g50670 | 1.41 |
| 1293 | 1294 | helicase-like protein | 1E-108 | At5g44800 | 1.5 |
| 1295 | 1296 | dynamin-like protein 4 (ADL4) | 1E-100 | At3g60190 | 1.32 |
| 1297 | 1298 | unknown protein | 0 | At3g12790 | 1.31 |
| 1299 | 1300 | putative Tub family protein | 0 | At2g47900 | 1.37 |
| 1301 | 1302 | putative protein | 1E-119 | At5g13020 | 1.33 |
| 1303 | 1304 | alanine aminotransferase, putative | 1E-147 | At1g17290 | 1.36 |
| 1305 | 1306 | SCARECROW-like protein | 0 | At4g36710 | 1.49 |
| 1307 | 1308 | alpha galactosyltransferase-like protein | 0 | At3g62720 | 3.26 |
| 1309 | 1310 | putative protein | 0 | At4g31980 | 1.32 |
| 1311 | 1312 | putative protein | 1E-124 | At3g56480 | 1.34 |
| 1313 | 1314 | histone acetyltransferase HAT B | 0 | At5g56740 | 2.36 |
| 1315 | 1316 | putative phosphoribosyl pyrophosphate synthetase | 3E-97 | At2g44530 | 1.45 |
| 1317 | 1318 | AIG1 | 1E-130 | At1g33960 | 1.45 |
| 1319 | 1320 | hypothetical protein | 0 | At4g22190 | 1.69 |
| 1321 | 1322 | hypothetical protein | 0 | At1g26180 | 1.33 |
| 1323 | 1324 | putative protein | 4E-84 | At5g59000 | 1.61 |
| 1325 | 1326 | hypothetical protein | 0 | At2g27660 | 1.66 |
| 1327 | 1328 | unknown protein | 0 | At1g33400 | 1.38 |
| 1329 | 1330 | helicase-like protein | 0 | At5g44800 | 1.63 |
| 1331 | 1332 | putative protein | 0 | At5g44920 | 1.43 |
| 1333 | 1334 | putative RNA-binding protein | 0 | At1g22910 | 2.13 |
| 1335 | 1336 | meiosis specific - like protein | 0 | At5g02820 | 2.62 |
| 1337 | 1338 | isocitrate dehydrogenase - like protein | 0 | At5g14590 | 1.43 |
| 1339 | 1340 | hypothetical protein | 1E-139 | At1g15500 | 1.63 |
| 1341 | 1342 | putative protein | 3E-01 | At5g52270 | 1.38 |
| 1343 | 1344 | ABC transporter-like protein | 0 | At5g06530 | 1.63 |
| 1345 | 1346 | heat-shock protein 90, putative | 0 | At1g27640 | 1.48 |
| 1347 | 1348 | unknown protein | 0 | At3g07220 | 1.33 |
| 2713 | 2714 | large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase | | NP_051067 | 4.71 |
| 2715 | 2716 | ribosomal protein L33 | | NP_051080 | 3.54 |
| 2717 | 2718 | PSII I protein | | NP_051074 | 2.81 |
| 2719 | 2720 | ribosomal protein L2 | | NP_051099 | 2.61 |
| 2721 | 2722 | ATP-dependent protease subunit | | NP_051083 | 2.60 |
| 2723 | 2724 | cytochrome B6 | | NP_051088 | 2.55 |
| 2725 | 2726 | ATPase epsilon subunit | | NP_051065 | 2.17 |
| 2728 | 2729 | 26S ribosomal RNA protein | | NP_085475 | 2.87 |

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|---|---|---|---|---|---|
| 2729 | 2730 | GATA Zn-finger protein | | At3g16870 | 2.75 |
| 2731 | 2732 | unknown protein | | At5g53740 | 2.01 |
| 2733 | 2734 | putative glucosyltransferase | | At2g15480 | 2.15 |
| 2735 | 2736 | Anthocyaninless2 | | At4g00730 | 2.73 |
| 2737 | 2738 | pectate lyase-like protein | | At3g54920 | 2.13 |
| 2739 | 2740 | putative sterol dehydrogenase | | At2g43420 | 2.10 |

*** This accession number was replaced by a new annotation and called At1g36160

**Table 5: Arabidopsis genes 1.3 times (1/ratio) or more repressed in E2Fa/Dpa plants**

| SEQ ID NO | | Gene name | E-value | MIPS accession Number | Ratio |
|---|---|---|---|---|---|
| cDNA | PROT | | | | |
| 1349 | 1350 | putative glutathione peroxidase | 0 | At2g31570 | 0.51 |
| 1351 | 1352 | phenylalanine ammonia lyase (PAL1) | 0 | At2g37040 | 0.65 |
| 1353 | 1354 | unknown protein | 0 | At1g04040 | 0.62 |
| 1355 | 1356 | putative protein | 0 | At4g25340 | 0.52 |
| 1357 | 1358 | water channel - like protein | 1E-129 | At4g23400 | 0.7 |
| 1359 | 1360 | catalase | 0 | At4g35090 | 0.46 |
| 1361 | 1362 | stearoyl-ACP desaturase | 2E-11 | At2g43710 | 0.54 |
| 1363 | 1364 | putative oligopeptide transporter | 0 | At4g10770 | 0.37 |
| 1365 | 1366 | putative chloroplast 50S ribosomal protein L28 | 0 | At2g33450 | 0.73 |
| 1367 | 1368 | ferredoxin--NADP reductase precursor, putative | 0 | At1g20020 | 0.64 |
| 1369 | 1370 | 3-beta-hydroxysteroid dehydrogenase | 1E-44 | At2g26260 | 0.73 |
| 1371 | 1372 | putative alanine aminotransferase | 1E-127 | At1g70580 | 0.51 |
| 1373 | 1374 | hypothetical protein | 4E-99 | At1g56500 | 0.66 |
| 1375 | 1376 | putative protein | 0 | At5g21940 | 0.64 |
| 1377 | 1378 | putative protein | 1E-158 | At5g26970 | 0.7 |
| 1379 | 1380 | actin depolymerizing factor 4 - like protein | 0 | At5g59890 | 0.66 |
| 1381 | 1382 | hypothetical protein | 7E-72 | At3g45160 | 0.5 |
| 1383 | 1384 | transporter-like protein | 1E-07 | At3g53960 | 0.68 |
| 1385 | 1386 | nicotianamine synthase (dbj\|BAA74589.1) | 0 | At5g04950 | 0.35 |
| 1387 | 1388 | cytochrome P450 monooxygenase (CYP83A1) | 0 | At4g13770 | 0.39 |
| 1389 | 1390 | unknown protein | 0 | At2g29660 | 0.77 |
| 1391 | 1392 | hypothetical protein | 0 | At3g12580 | 0.56 |
| 1393 | 1394 | unknown protein | 0 | At5g64130 | 0.52 |
| 1395 | 1396 | putative protein | 0 | At3g61870 | 0.73 |
| 1397 | 1398 | fructose-bisphosphate aldolase - like protein | 0 | At4g26530 | 0.17 |
| 1399 | 1400 | lectin like protein | 1E-124 | At4g19840 | 0.74 |
| 1401 | 1402 | unknown protein | 0 | At1g28140 | 0.72 |
| 1403 | 1404 | feebly-like protein | 0 | At3g01420 | 0.73 |
| 1405 | 1406 | beta-fructosidase | 1E-105 | At1g62660 | 0.38 |
| 1407 | 1408 | unknown protein | 1E-06 | At1g15350 | 0.77 |
| 1409 | 1410 | peptidylprolyl isomerase ROC1 | 0 | At4g38740 | 0.76 |
| 1411 | 1412 | hypothetical protein | 1E-36 | At2g06010 | 0.74 |
| 1413 | 1414 | putative protein | 1E-114 | At4g30490 | 0.5 |
| 1415 | 1416 | 3-isopropylmalate dehydrogenase | 0 | At5g14200 | 0.61 |
| 1417 | 1418 | putative copper/zinc superoxide dismutase | 1E-93 | At2g28190 | 0.77 |
| 1419 | 1420 | putative myo-inositol 1-phosphate synthase | 0 | At2g22240 | 0.68 |
| 1421 | 1422 | putative enolase (2-phospho-D-glycerate hydroylase) | 0 | At2g29560 | 0.7 |
| 1423 | 1424 | unknown protein | 0 | At5g43750 | 0.4 |
| 1425 | 1426 | putative protein | 1E-22 | At4g32330 | 0.68 |

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|---|---|---|---|---|---|
| 1427 | 1428 | putative ferredoxin-thioredoxin reductase | 0 | At2g04700 | 0.75 |
| 1429 | 1430 | hypothetical protein | 1E+00 | At3g23290 | 0.59 |
| 1431 | 1432 | putative cellulose synthase | 0 | At2g32530 | 0.58 |
| 1433 | 1434 | putative protein | 0 | At5g43850 | 0.54 |
| 1435 | 1436 | putative protein | 0 | At5g03010 | 0.58 |
| 1437 | 1438 | hypothetical protein | 0 | At1g78140 | 0.61 |
| 1439 | 1440 | unknown protein | 0 | At1g72590 | 0.35 |
| 1441 | 1442 | hypothetical protein | 0 | At1g54450 | 0.59 |
| 1443 | 1444 | hypothetical protein | 0 | At1g19110 | 0.73 |
| 1445 | 1446 | endo-beta-1,4-glucanase, putative | 0 | At1g75680 | 0.7 |
| 1447 | 1448 | unknown protein | 0 | At1g63010 | 0.76 |
| 1449 | 1450 | hypothetical protein | 2E-58 | At4g24700 | 0.57 |
| 1451 | 1452 | glyoxalase II | 0 | At1g53580 | 0.65 |
| 1453 | 1454 | putative protein | 0 | At3g52370 | 0.53 |
| 1455 | 1456 | unknown protein | 0 | At1g80280 | 0.57 |
| 1457 | 1458 | protein phosphatase ABI1 | 0 | At4g26080 | 0.71 |
| 1459 | 1460 | 33 kDa polypeptide of oxygen-evolving complex (OEC) in photosystem | 1E-115 | At5g66570 | 0.65 |
| 1461 | 1462 | beta-xylosidase | 1E-163 | At5g64570 | 0.55 |
| 1463 | 1464 | GDP-mannose pyrophosphorylase | 0 | At2g39770 | 0.62 |
| 1465 | 1466 | peroxidase ATP20a (emb\|CAA67338.1) | 0 | At5g14130 | 0.67 |
| 1467 | 1468 | putative glutathione transferase | 0 | At1g17190 | 0.71 |
| 1469 | 1470 | putative protein | 0 | At4g38080 | 0.75 |
| 1471 | 1472 | unknown protein | 1E-179 | At1g61190 | 0.7 |
| 1473 | 1474 | 50S ribosomal protein L24, chloroplast precursor | 0 | At5g54600 | 0.76 |
| 1475 | 1476 | unknown protein | 1E-179 | At1g68260 | 0.55 |
| 1477 | 1478 | subtilisin-like serine proteinase, putative, 3' partial | 0 | At3g14067 | 0.62 |
| 1479 | 1480 | putative protein | 0 | At4g23890 | 0.59 |
| 1481 | 1482 | unknown protein | 0 | At3g01690 | 0.7 |
| 1483 | 1484 | putative protein | 0 | At3g56290 | 0.3 |
| 1485 | 1486 | unknown protein | 0 | At2g39450 | 0.67 |
| 1487 | 1488 | unknown protein | 0 | At5g64130 | 0.66 |
| 1489 | 1490 | putative protein | 0 | At4g30140 | 0.54 |
| 1491 | 1492 | ribulose bisphosphate carboxylase small chain 3b precursor (RuBisCO | 1E-145 | At5g38410 | 0.54 |
| 1493 | 1494 | Myb DNA binding protein -like | 0 | At3g46130 | 0.75 |
| 1495 | 1496 | putative 2-cys peroxiredoxin | 0 | At3g11630 | 0.64 |
| 1497 | 1498 | putative trypsin inhibitor | 0 | At1g73260 | 0.59 |
| 1499 | 1500 | O-methyltransferase | 1E-127 | At5g54160 | 0.62 |
| 1501 | 1502 | hypothetical protein | 2E-30 | At1g29270 | 0.73 |
| 1503 | 1504 | RP19 gene for chloroplast ribosomal protein CL9 | 9E-67 | At3g44890 | 0.68 |
| 1505 | 1506 | putative phosphoglyceride transfer protein | 1E-178 | At4g08690 | 0.57 |
| 1507 | 1508 | putative protein | 0 | At5g63530 | 0.53 |
| 1509 | 1510 | putative protein | 0 | At5g38720 | 0.68 |
| 1511 | 1512 | hypothetical protein | 0 | At1g72030 | 0.68 |
| 1513 | 1514 | unknown protein | 9E-21 | At5g09990 | 0.67 |
| 1515 | 1516 | zinc finger protein ZAT7 | 0 | At3g46090 | 0.73 |
| 1517 | 1518 | putative nodulin | 0 | At3g05180 | 0.64 |
| 1519 | 1520 | putative wound-induced basic protein | 1E-160 | At3g07230 | 0.75 |
| 1521 | 1522 | hypothetical protein | 0 | At4g02920 | 0.38 |
| 1523 | 1524 | putative protein | 1E-154 | At5g62220 | 0.73 |
| 1525 | 1526 | myosin heavy chain-like protein | 0 | At3g16000 | 0.5 |

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|---|---|---|---|---|---|
| 1527 | 1528 | unknown protein | 0 | At1g09610 | 0.76 |
| 1529 | 1530 | arabinogalactan protein - like | 0 | At5g03170 | 0.71 |
| 1531 | 1532 | biotin carboxyl carrier protein of acetyl-CoA carboxylase precursor | 0 | At5g16390 | 0.69 |
| 1533 | 1534 | centrin | 0 | At3g50360 | 0.74 |
| 1535 | 1536 | vegetative storage protein Vsp1 | 0 | At5g24780 | 0.48 |
| 1537 | 1538 | protein kinase, putative | 1E-61 | At1g52310 | 0.63 |
| 1539 | 1540 | unknown protein | 1E-132 | At2g42760 | 0.63 |
| 1541 | 1542 | phenylalanine ammonia lyase (PAL1) | 0 | At2g37040 | 0.72 |
| 1543 | 1544 | UDP rhamnose-anthocyanidin-3-glucoside rhamnosyltransferase - like | 0 | At4g27560 | 0.45 |
| 1545 | 1546 | unknown protein | 0 | At2g17500 | 0.54 |
| 1547 | 1548 | NAC domain protein, putative | 0 | At1g01720 | 0.72 |
| 1549 | 1550 | ubiquitin-conjugating enzyme-like protein | 2E-24 | At5g56150 | 0.41 |
| 1551 | 1552 | putative RNA-binding protein | 1E-136 | At2g37220 | 0.72 |
| 1553 | 1554 | Overlap with bases 87,142-90,425 of 'IGF' BAC clone F9K20, accession | 0 | At1g78570 | 0.52 |
| 1555 | 1556 | hypothetical protein | 1E-105 | At2g04040 | 0.52 |
| 1557 | 1558 | Isp4-like protein | 4E-01 | At5g64410 | 0.39 |
| 1559 | 1560 | ids4-like protein | 0 | At5g20150 | 0.58 |
| 1561 | 1562 | unknown protein | 3E-98 | At1g44000 | 0.67 |
| 1563 | 1564 | R2R3-MYB transcription factor | 0 | At3g50060 | 0.66 |
| 1565 | 1566 | putative hexose transporter | 0 | At4g02050 | 0.68 |
| 1567 | 1568 | one helix protein (OHP) | 0 | At5g02120 | 0.57 |
| 1569 | 1570 | UDP-glucose dehydrogenase-like protein | 0 | At5g15490 | 0.74 |
| 1571 | 1572 | putative protein | 0 | At3g54260 | 0.63 |
| 1573 | 1574 | putative L5 ribosomal protein | 0 | At4g01310 | 0.75 |
| 1575 | 1576 | putative myosin heavy chain | 0 | At2g37080 | 0.61 |
| 1577 | 1578 | clpB heat shock protein-like | 0 | At5g15450 | 0.57 |
| 1579 | 1580 | unknown protein | 4E-71 | At1g52510 | 0.66 |
| 1581 | 1582 | beta-fructosidase, putative | 0 | At1g12240 | 0.55 |
| 1583 | 1584 | hypothetical protein | 0 | At1g47670 | 0.69 |
| 1585 | 1586 | putative protein | 3E-36 | At5g25890 | 0.75 |
| 1587 | 1588 | predicted protein | 1E-108 | At4g31390 | 0.73 |
| 1589 | 1590 | putative phospholipase | 0 | At2g39420 | 0.66 |
| 1591 | 1592 | ATP-dependent transmembrane transporter, putative | 0 | At1g51460 | 0.74 |
| 1593 | 1594 | H+-transporting ATP synthase-like protein | 0 | At4g09650 | 0.64 |
| 1595 | 1596 | putative protein | 0 | At4g29590 | 0.77 |
| 1597 | 1598 | unknown protein | 0 | At3g02640 | 0.49 |
| 1599 | 1600 | phosphoenolpyruvate carboxylase (PPC) | 0 | At3g14940 | 0.77 |
| 1601 | 1602 | pollen allergen-like protein | 0 | At1g24020 | 0.28 |
| 1603 | 1604 | putative AUX1-like permease | 0 | At1g77690 | 0.73 |
| 1605 | 1606 | putative protein | 1E-127 | At4g39730 | 0.49 |
| 1607 | 1608 | homeobox-leucine zipper protein ATHB-12 | 0 | At3g61890 | 0.24 |
| 1609 | 1610 | putative protein | 0 | At5g10160 | 0.53 |
| 1611 | 1612 | unknown protein | 0 | At1g71480 | 0.56 |
| 1613 | 1614 | putative violaxanthin de-epoxidase precursor (U44133) | 0 | At1g08550 | 0.7 |
| 1615 | 1616 | nClpP5, putative | 0 | At1g49970 | 0.68 |
| 1617 | 1618 | hypothetical protein | 0 | At1g65260 | 0.57 |
| 1619 | 1620 | putative protein | 1E-135 | At3g52360 | 0.38 |
| 1621 | 1622 | putative protein | 0 | At5g26260 | 0.5 |
| 1623 | 1624 | unknown protein | 0 | At1g25170 | 0.66 |
| 1625 | 1626 | hypothetical protein | 0 | At1g79550 | 0.65 |

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|---|---|---|---|---|---|
| 1627 | 1628 | tubulin beta-2/beta-3 chain (sp\|P29512) | 2E-21 | At5g62700 | 0.61 |
| 1629 | 1630 | eukaryotic translation initiation factor 4E, putative | 0 | At1g29550 | 0.64 |
| 1631 | 1632 | transport inhibitor response 1, putative | 1E-175 | At1g12820 | 0.77 |
| 1633 | 1634 | osmotin precursor | 1E-110 | At4g11650 | 0.74 |
| 1635 | 1636 | putative glutathione S-transferase TSI-1 | 0 | At1g10360 | 0.72 |
| 1637 | 1638 | protein ch-42 precursor, chloroplast | 0 | At4g18480 | 0.76 |
| 1639 | 1640 | omega-3 fatty acid desaturase | 2E-06 | At2g29980 | 0.73 |
| 1641 | 1642 | unknown protein | 0 | At2g44670 | 0.57 |
| 1643 | 1644 | putative protein | 0 | At3g55330 | 0.51 |
| 1645 | 1646 | putative calmodulin | 0 | At3g51920 | 0.55 |
| 1647 | 1648 | plastid ribosomal protein L34 precursor, putative | 1E-140 | At1g29070 | 0.69 |
| 1649 | 1650 | putative protein | 0 | At5g67070 | 0.66 |
| 1651 | 1652 | putative 2Fe-2S iron-sulfur cluster protein | 0 | At3g16250 | 0.69 |
| 1653 | 1654 | hypothetical protein | 0 | At1g42970 | 0.69 |
| 1655 | 1656 | hypothetical protein | 3E-69 | At3g14190 | 0.6 |
| 1657 | 1658 | thylakoid luminal protein | 1E-122 | At1g77090 | 0.7 |
| 1659 | 1660 | putative protein | 0 | At3g48420 | 0.42 |
| 1661 | 1662 | actin 3 | 0 | At2g37620 | 0.64 |
| 1663 | 1664 | OEP8 like protein | 4E-38 | At4g15800 | 0.73 |
| 1665 | 1666 | putative Ras-like GTP-binding protein | 0 | At3g09910 | 0.71 |
| 1667 | 1668 | sulfolipid biosynthesis protein SQD1 | 0 | At4g33030 | 0.68 |
| 1669 | 1670 | oleosin isoform | 0 | At3g27660 | 0.61 |
| 1671 | 1672 | acyl-CoA synthetase, putative | 0 | At1g64400 | 0.59 |
| 1673 | 1674 | putative protein | 1E-147 | At3g61060 | 0.5 |
| 1675 | 1676 | hypothetical protein | 1E-117 | At1g56200 | 0.64 |
| 1677 | 1678 | putative protein | 0 | At4g13500 | 0.53 |
| 1679 | 1680 | cinnamoyl CoA reductase, putative | 0 | At1g80820 | 0.72 |
| 1681 | 1682 | hypothetical protein | 1E-157 | At4g28410 | 0.1 |
| 1683 | 1684 | hypothetical protein | 0 | At1g54030 | 0.68 |
| 1685 | 1686 | putative DNA-binding protein, GT-1 | 0 | At3g25990 | 0.1 |
| 1687 | 1688 | germin-like protein | 3E-04 | At3g05950 | 0.49 |
| 1689 | 1690 | putative glutathione S-transferase | 0E+00 | At2g29480 | 0.7 |
| 1691 | 1692 | arabinogalactan-protein (gb\|AAC77823.1) | 1E-06 | At5g64310 | 0.61 |
| 1693 | 1694 | periaxin - like protein | 1E-151 | At5g09530 | 0.71 |
| 1695 | 1696 | zeaxanthin epoxidase precursor | 0 | At5g67030 | 0.52 |
| 1697 | 1698 | putative photosystem I reaction center subunit IV | 0 | At2g20260 | 0.7 |
| 1699 | 1700 | putative 60S ribosomal protein L18A | 0 | At3g14600 | 0.74 |
| 1701 | 1702 | putative ethylene response element binding protein (EREBP) | 0 | At2g44840 | 0.72 |
| 1703 | 1704 | unknown protein | 0 | At2g21970 | 0.5 |
| 1705 | 1706 | RNA-binding protein cp33 precursor | 0 | At3g52380 | 0.73 |
| 1707 | 1708 | unknown protein | 1E-152 | At2g34460 | 0.62 |
| 1709 | 1710 | CONSTANS-like 1 | 1E-179 | At5g15850 | 0.6 |
| 1711 | 1712 | unknown protein | 0 | At1g75100 | 0.77 |
| 1713 | 1714 | ion channel | 9E-66 | At1g15990 | 0.57 |
| 1715 | 1716 | unknown protein | 0 | At2g21960 | 0.46 |
| 1717 | 1718 | unknown protein | 0 | At1g66330 | 0.69 |
| 1719 | 1720 | putative protein | 0 | At4g26630 | 0.68 |
| 1721 | 1722 | unknown protein | 1E-99 | At3g28230 | 0.72 |
| 1723 | 1724 | hypothetical protein | 1E-65 | At1g55910 | 0.65 |
| 1725 | 1726 | putative Na+-dependent inorganic phosphate cotransporter | 0 | At2g29650 | 0.52 |
| 1727 | 1728 | hypothetical protein | 4E-23 | At1g02330 | 0.71 |
| 1729 | 1730 | hypothetical protein | 0 | At1g29700 | 0.55 |

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|---|---|---|---|---|---|
| 1731 | 1732 | putative flavonol 3-O-glucosyltransferase | 0 | At2g18560 | 0.62 |
| 1733 | 1734 | lycopene epsilon cyclase | 0 | At5g57030 | 0.6 |
| 1735 | 1736 | hypothetical protein | 0 | At3g09150 | 0.75 |
| 1737 | 1738 | putative protein | 1E-150 | At1g31710 | 0.5 |
| 1739 | 1740 | hypothetical protein | 0 | At1g78850 | 0.69 |
| 1741 | 1742 | putative protein | 0 | At4g32770 | 0.75 |
| 1743 | 1744 | putative protein | 2E-77 | At4g22890 | 0.75 |
| 1745 | 1746 | ripening-related protein - like | 0 | At5g20740 | 0.59 |
| 1747 | 1748 | putative peroxidase ATP12a | 0 | At1g05240 | 0.65 |
| 1749 | 1750 | hypothetical protein | 7E-18 | At4g01050 | 0.77 |
| 1751 | 1752 | V-ATPase subunit G (vag2 gene) | 4E-04 | At4g23710 | 0.61 |
| 1753 | 1754 | hypothetical protein | 0 | At1g58080 | 0.75 |
| 1755 | 1756 | putative protein | 2E-94 | At5g19190 | 0.51 |
| 1757 | 1758 | hypothetical protein | 0 | At1g48850 | 0.69 |
| 1759 | 1760 | putative protein | 0 | At4g38800 | 0.75 |
| 1761 | 1762 | similar to polygalacturonase-like protein | 0 | At1g10640 | 0.28 |
| 1763 | 1764 | putative glutathione S-transferase | 0 | At2g02390 | 0.73 |
| 1765 | 1766 | putative calcium-binding EF-hand protein | 3E-78 | At2g33380 | 0.69 |
| 1767 | 1768 | unknown protein | 1E-113 | At1g64680 | 0.57 |
| 1769 | 1770 | unknown protein | 0 | At3g15660 | 0.58 |
| 1771 | 1772 | putative protein | 0 | At5g22080 | 0.74 |
| 1773 | 1774 | high mobility group protein 2-like | 2E-24 | At3g51880 | 0.71 |
| 1775 | 1776 | similar to late embryogenesis abundant proteins | 4E-50 | At2g44060 | 0.61 |
| 1777 | 1778 | putative protein | 0 | At4g34600 | 0.74 |
| 1779 | 1780 | putative protein | 2E-31 | At5g52060 | 0.48 |
| 1781 | 1782 | NADPH oxidoreductase, putative | 0 | At1g75280 | 0.53 |
| 1783 | 1784 | hypothetical protein | 0 | At1g16720 | 0.62 |
| 1785 | 1786 | unknown protein | 0 | At3g28130 | 0.75 |
| 1787 | 1788 | glutaredoxin | 0 | At4g15690 | 0.73 |
| 1789 | 1790 | putative protein | 4E-01 | At3g47590 | 0.66 |
| 1791 | 1792 | putative protein | 0 | At4g26630 | 0.7 |
| 1793 | 1794 | putative polyprotein | 1E-139 | At4g04410 | 0.76 |
| 1795 | 1796 | MTN3-like protein | 0 | At3g48740 | 0.49 |
| 1797 | 1798 | hypothetical protein | 0 | At1g32900 | 0.38 |
| 1799 | 1800 | unknown protein | 0 | At2g33180 | 0.77 |
| 1801 | 1802 | hypothetical protein | 0 | At1g66890 | 0.69 |
| 1803 | 1804 | unknown protein | 0 | At1g74730 | 0.74 |
| 1805 | 1806 | putative ribosomal protein S9 | 1E-122 | At1g74970 | 0.7 |
| 1807 | 1808 | phenylalanine ammonia-lyase | 3E-51 | At3g53260 | 0.53 |
| 1809 | 1810 | unknown protein | 2E-27 | At1g78110 | 0.76 |
| 1811 | 1812 | unknown protein | 0 | At1g18300 | 0.75 |
| 1813 | 1814 | putative prolylcarboxypeptidase | 1E-174 | At2g24280 | 0.64 |
| 1815 | 1816 | unknown protein | 1E-12 | At3g24100 | 0.76 |
| 1817 | 1818 | unknown protein | 0 | At3g18990 | 0.39 |
| 1819 | 1820 | hypothetical protein | 1E-127 | At1g78890 | 0.75 |
| 1821 | 1822 | unknown protein | 5E-87 | At2g21530 | 0.71 |
| 1823 | 1824 | hypothetical protein | 1E-172 | At1g20340 | 0.71 |
| 1825 | 1826 | putative glucosyltransferase | 0 | At2g31790 | 0.63 |
| 1827 | 1828 | allergen like protein | 1E-129 | At4g17030 | 0.74 |
| 1829 | 1830 | unknown protein | 0 | At1g73750 | 0.72 |
| 1831 | 1832 | APG5 (autophagy 5)-like protein | 0 | At5g17290 | 0.7 |
| 1833 | 1834 | putative protochlorophyllide reductase | 0 | At1g03630 | 0.57 |

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|---|---|---|---|---|---|
| 1835 | 1836 | zinc finger protein, putative | 0 | At3g19580 | 0.61 |
| 1837 | 1838 | unknown protein | 0 | At2g35190 | 0.65 |
| 1839 | 1840 | phosphate/triose-phosphate translocator precursor (gb\|AAC83815.1) | 4E-33 | At5g46110 | 0.73 |
| 1841 | 1842 | unknown protein | 0 | At5g50840 | 0.77 |
| 1843 | 1844 | hypothetical protein | 0 | At4g34090 | 0.69 |
| 1845 | 1846 | hypothetical protein | 0 | At1g14340 | 0.64 |
| 1847 | 1848 | unknown protein | 0 | At1g67860 | 0.42 |
| 1849 | 1850 | tyrosine transaminase like protein | 1E-180 | At4g23600 | 0.47 |
| 1851 | 1852 | unknown protein | 1E-173 | At1g53890 | 0.53 |
| 1853 | 1854 | pectinesterase, putative | 0 | At1g41830 | 0.76 |
| 1855 | 1856 | putative protein | 4E-72 | At5g45550 | 0.69 |
| 1857 | 1858 | putative ligand-gated ion channel subunit | 2E+00 | At2g32400 | 0.45 |
| 1859 | 1860 | unknown protein | 0 | At3g19370 | 0.42 |
| 1861 | 1862 | putative protein | 5E-13 | At5g62580 | 0.59 |
| 1863 | 1864 | putative protein | 0 | At3g61080 | 0.42 |
| 1865 | 1866 | putative squamosa-promoter binding protein 2 | 1E-162 | At1g27360 | 0.74 |
| 1867 | 1868 | sucrose-phosphate synthase - like protein | 0 | At4g10120 | 0.22 |
| 1869 | 1870 | hypothetical protein | 4E-23 | At1g62180 | 0.43 |
| 1871 | 1872 | ribosomal protein | 0 | At4g15000 | 0.75 |
| 1873 | 1874 | MYB-related transcription factor (CCA1) | 0 | At2g46830 | 0.46 |
| 1875 | 1876 | pinoresinol-lariciresinol reductase, putative | 1E-124 | At1g32100 | 0.72 |
| 1877 | 1878 | putative protein | 0 | At3g52230 | 0.71 |
| 1879 | 1880 | 3-keto-acyl-CoA thiolase 2 (gb\|AAC17877.1) | 0 | At5g48880 | 0.57 |
| 1881 | 1882 | putative protein | 0 | At3g46780 | 0.63 |
| 1883 | 1884 | DNA-binding protein, putative | 0 | At1g01060 | 0.62 |
| 1885 | 1886 | putative protein | 3E-20 | At4g30990 | 0.6 |
| 1887 | 1888 | putative protein | 0 | At3g46780 | 0.59 |
| 1889 | 1890 | hypothetical protein | 1E-174 | At1g28400 | 0.58 |
| 1891 | 1892 | DNA binding protein - like | 0 | At5g61600 | 0.55 |
| 1893 | 1894 | putative protein | 0 | At3g62260 | 0.72 |
| 1895 | 1896 | putative CCCH-type zinc finger protein | 0 | At2g25900 | 0.63 |
| 1897 | 1898 | ubiquitin-conjugating enzyme E2-17 kD 8 (ubiquitin-protein ligase | 3E-16 | At5g41700 | 0.42 |
| 1899 | 1900 | microbody NAD-dependent malate dehydrogenase | 0 | At5g09660 | 0.63 |
| 1901 | 1902 | glyceraldehyde 3-phosphate dehydrogenase A subunit (GapA) | 0 | At3g26650 | 0.63 |
| 1903 | 1904 | microbody NAD-dependent malate dehydrogenase | 0 | At5g09660 | 0.66 |
| 1905 | 1906 | sedoheptulose-bisphosphatase precursor | 0 | At3g55800 | 0.54 |
| 1907 | 1908 | putative Fe(II) transporter | 1E-175 | At2g32270 | 0.74 |
| 1909 | 1910 | germin - like protein | 0 | At5g38940 | 0.75 |
| 1911 | 1912 | putative malonyl-CoA:Acyl carrier protein transacylase | 0 | At2g30200 | 0.7 |
| 1913 | 1914 | hypothetical protein | 0 | At1g19000 | 0.61 |
| 1915 | 1916 | FRO1-like protein; NADPH oxidase-like | 0 | At5g49740 | 0.41 |
| 1917 | 1918 | J8-like protein | 0 | At1g80920 | 0.51 |
| 1919 | 1920 | putative protein | 0 | At4g34190 | 0.63 |
| 1921 | 1922 | photosystem II stability/assembly factor HCF136 (sp\|O82660) | 0 | At5g23120 | 0.66 |
| 1923 | 1924 | hypothetical protein | 0 | At4g24930 | 0.63 |
| 1925 | 1926 | 2-cys peroxiredoxin-like protein | 0 | At5g06290 | 0.69 |
| 1927 | 1928 | putative protein | 0 | At3g53470 | 0.54 |
| 1929 | 1930 | unknown protein . | 3E-96 | At3g02180 | 0.71 |
| 1931 | 1932 | F12P19.7 | 0 | At1g65900 | 0.69 |
| 1933 | 1934 | putative fibrillin | 0 | At4g04020 | 0.28 |
| 1935 | 1936 | putative protein | 1E-01 | At4g18810 | 0.72 |

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|---|---|---|---|---|---|
| 1937 | 1938 | hypothetical protein | 1E-171 | At1g50240 | 0.67 |
| 1939 | 1940 | putative protein | 0 | At3g63210 | 0.76 |
| 1941 | 1942 | unknown protein | 0 | At2g32870 | 0.47 |
| 1943 | 1944 | Glucose-1-phosphate adenylyltransferase (ApL1/adg2) | 0 | At5g19220 | 0.64 |
| 1945 | 1946 | unknown protein | 1E-66 | At2g46100 | 0.67 |
| 1947 | 1948 | farnesyl diphosphate synthase precursor (gb\|AAB49290.1) | 0 | At5g47770 | 0.71 |
| 1949 | 1950 | pyridoxine biosynthesis protein - like | 0 | At5g01410 | 0.47 |
| 1951 | 1952 | hypothetical protein | 0 | At4g03820 | 0.71 |
| 1953 | 1954 | putative myrosinase-binding protein | 1E-47 | At2g39310 | 0.38 |
| 1955 | 1956 | unknown protein | 0 | At1g05870 | 0.44 |
| 1957 | 1958 | heat shock protein, putative | 0 | At1g06460 | 0.28 |
| 1959 | 1960 | RIBOSOMAL PROTEIN, putative | 1E-175 | At1g71720 | 0.76 |
| 1961 | 1962 | elongation factor G, putative | 0 | At1g62750 | 0.65 |
| 1963 | 1964 | mitochondrial Lon protease homolog 1 precursor (sp\|O64948) | 0 | At5g47040 | 0.76 |
| 1965 | 1966 | cytochrome c | 2E-37 | At4g10040 | 0.72 |
| 1967 | 1968 | hypothetical protein | 1E-102 | At4g03420 | 0.69 |
| 1969 | 1970 | putative DnaJ protein | 1E-160 | At2g41000 | 0.73 |
| 1971 | 1972 | hypothetical protein | 0 | At2g27290 | 0.61 |
| 1973 | 1974 | putative protein | 1E-117 | At5g50100 | 0.4 |
| 1975 | 1976 | phytoene synthase (gb\|AAB65697.1) | 0 | At5g17230 | 0.64 |
| 1977 | 1978 | putative protein | 0 | At4g28230 | 0.73 |
| 1979 | 1980 | hypothetical protein | 0 | At2g01260 | 0.49 |
| 1981 | 1982 | unknown protein | 0 | At3g17520 | 0.71 |
| 1983 | 1984 | Ran binding protein (AtRanBP1b) | 0 | At2g30060 | 0.73 |
| 1985 | 1986 | putative protein | 0 | At4g32190 | 0.63 |
| 1987 | 1988 | unknown protein | 0 | At1g19400 | 0.64 |
| 1989 | 1990 | sucrose-phosphate synthase-like protein | 0 | At5g20280 | 0.67 |
| 1991 | 1992 | putative protein | 1E-136 | At5g03545 | 0.45 |
| 1993 | 1994 | biotin carboxyl carrier protein precursor-like protein | 1E-124 | At5g15530 | 0.54 |
| 1995 | 1996 | unknown protein | 4E-85 | At1g16320 | 0.53 |
| 1997 | 1998 | unknown protein | 5E-16 | At3g32930 | 0.68 |
| 1999 | 2000 | putative protein | 1E-142 | At4g35290 | 0.74 |
| 2001 | 2002 | glutathione S-transferase-like protein | 0 | At5g17220 | 0.66 |
| 2003 | 2004 | fructose 1,6-bisphosphatase, putative | 0 | At1g43670 | 0.63 |
| 2005 | 2006 | peptidylprolyl isomerase-like protein | 2E-34 | At5g13120 | 0.72 |
| 2007 | 2008 | teosinte branched1 - like protein | 0 | At4g18390 | 0.63 |
| 2009 | 2010 | putative protein | 0 | At3g51520 | 0.71 |
| 2011 | 2012 | lactoylglutathione lyase-like protein | 0 | At1g11840 | 0.45 |
| 2013 | 2014 | late embryogenesis abundant protein LEA like | 0 | At5g06760 | 0.55 |
| 2015 | 2016 | putative protein | 1E-177 | At5g19590 | 0.71 |
| 2017 | 2018 | putative protein | 0 | At3g63190 | 0.72 |
| 2019 | 2020 | hypothetical protein | 0 | At1g69510 | 0.47 |
| 2021 | 2022 | putative protein kinase | 0 | At2g30040 | 0.69 |
| 2023 | 2024 | xyloglucan endo-transglycosylase | 0 | At3g44990 | 0.43 |
| 2025 | 2026 | phospholipid hydroperoxide glutathione peroxidase | 0 | At4g11600 | 0.71 |
| 2027 | 2028 | sedoheptulose-bisphosphatase precursor | 0 | At3g55800 | 0.51 |
| 2029 | 2030 | Clp proteinase like protein | 2E-55 | At4g17040 | 0.75 |
| 2031 | 2032 | unknown protein | 0 | At5g07020 | 0.68 |
| 2033 | 2034 | unknown protein | 2E-32 | At5g51720 | 0.49 |
| 2035 | 2036 | endomembrane protein, putative | 1E-117 | At1g14670 | 0.75 |
| 2037 | 2038 | putative phosphomannomutase | 0 | At2g45790 | 0.66 |
| 2039 | 2040 | putative protein | 1E-95 | At4g27280 | 0.46 |

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|---|---|---|---|---|---|
| 2041 | 2042 | mrp protein, putative | 0 | At3g24430 | 0.75 |
| 2043 | 2044 | putative vacuolar ATPase | 0 | At4g02620 | 0.74 |
| 2045 | 2046 | phosphate transporter, putative | 0 | At3g26570 | 0.61 |
| 2047 | 2048 | similar to Trp Asp repeat protein emb\|CAB39845.1; similar to EST | 0 | At1g78070 | 0.74 |
| 2049 | 2050 | putative MAP kinase | 2E-18 | At2g01450 | 0.51 |
| 2051 | 2052 | ethylene-responsive transcriptional coactivator, putative | 0 | At3g24500 | 0.51 |
| 2053 | 2054 | 6-phosphogluconolactonase-like protein | 0 | At5g24420 | 0.52 |
| 2055 | 2056 | beta-amylase-like proten | 1E-175 | At5g18670 | 0.4 |
| 2057 | 2058 | hypothetical protein | 3E-53 | At1g20970 | 0.72 |
| 2059 | 2060 | chloroplast 50S ribosomal protein L31, putative | 0 | At1g75350 | 0.74 |
| 2061 | 2062 | cytochrome P450-like protein | 0 | At4g37320 | 0.67 |
| 2063 | 2064 | putative potassium transporter AtKT5p (AtKT5) | 0 | At4g33530 | 0.76 |
| 2065 | 2066 | putative ribosomal-protein S6 kinase (ATPK6) | 0 | At3g08730 | 0.63 |
| 2067 | 2068 | hypothetical protein | 0 | At1g04770 | 0.68 |
| 2069 | 2070 | transcription factor Hap5a | 6E-74 | At3g48590 | 0.6 |
| 2071 | 2072 | putative protein | 0 | At5g20070 | 0.69 |
| 2073 | 2074 | beta-expansin | 0 | At2g20750 | 0.72 |
| 2075 | 2076 | SOUL-like protein | 4E-82 | At1g17100 | 0.71 |
| 2077 | 2078 | unknown protein | 0 | At1g70760 | 0.4 |
| 2079 | 2080 | unknown protein | 1E-124 | At2g20890 | 0.73 |
| 2081 | 2082 | unknown protein | 1E-160 | At1g07280 | 0.72 |
| 2083 | 2084 | unknown protein | 0 | At1g64680 | 0.65 |
| 2085 | 2086 | ADPG pyrophosphorylase small subunit (gb\|AAC39441.1) | 0 | At5g48300 | 0.68 |
| 2087 | 2088 | unknown protein | 0 | At2g17340 | 0.61 |
| 2089 | 2090 | hypothetical protein | 0 | At1g26800 | 0.74 |
| 2091 | 2092 | unknown protein | 0 | At1g22930 | 0.67 |
| 2093 | 2094 | polyphosphoinositide binding protein, putative | 0 | At1g01630 | 0.72 |
| 2095 | 2096 | caffeoyl-CoA O-methyltransferase - like protein | 0 | At4g34050 | 0.67 |
| 2097 | 2098 | pectinesterase | 0 | At5g53370 | 0.56 |
| 2099 | 2100 | unknown protein | 7E-75 | At1g64370 | 0.43 |
| 2101 | 2102 | p-nitrophenylphosphatase-like protein | 0 | At5g36790 | 0.52 |
| 2103 | 2104 | putative protein | 1E-172 | At5g55960 | 0.64 |
| 2105 | 2106 | serine/threonine protein kinase -like protein | 0 | At5g10930 | 0.26 |
| 2107 | 2108 | cytosolic factor, putative | 0 | At1g72160 | 0.67 |
| 2109 | 2110 | S-adenosylmethionine:2-demethylmenaquinone methyltransferase-like | 1E-159 | At5g56260 | 0.76 |
| 2111 | 2112 | pectate lyase | 0 | At5g63180 | 0.67 |
| 2113 | 2114 | vacuolar sorting receptor-like protein | 0 | At4g20110 | 0.7 |
| 2115 | 2116 | putative membrane channel protein | 0 | At2g28900 | 0.76 |
| 2117 | 2118 | putative thylakoid lumen rotamase | 0 | At3g01480 | 0.56 |
| 2119 | 2120 | putative chloroplast prephenate dehydratase | 0 | At3g44720 | 0.73 |
| 2121 | 2122 | 3-oxoacyl-[acyl-carrier-protein] synthase I precursor | 0 | At5g46290 | 0.76 |
| 2123 | 2124 | P-Protein - like protein | 1E-108 | At4g33010 | 0.73 |
| 2125 | 2126 | NHE1 Na+/H+ exchanger | 1E-122 | At5g27150 | 0.73 |
| 2127 | 2128 | receptor kinase-like protein | 0 | At3g47580 | 0.72 |
| 2129 | 2130 | raffinose synthase -like protein | 0 | At5g40390 | 0.59 |
| 2131 | 2132 | thylakoid luminal protein | 0 | At1g54780 | 0.63 |
| 2133 | 2134 | unknown protein | 0 | At2g46170 | 0.73 |
| 2135 | 2136 | beta-xylan endohydrolase -like protein | 9E-02 | At4g33810 | 0.26 |
| 2137 | 2138 | putative protein | 1E-137 | At4g12700 | 0.6 |
| 2139 | 2140 | putative ribose 5-phosphate isomerase | 0 | At3g04790 | 0.76 |
| 2141 | 2142 | putative protein | 0 | At5g47840 | 0.7 |

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|---|---|---|---|---|---|
| 2143 | 2144 | putative RNA-binding protein | 0 | At1g09340 | 0.57 |
| 2145 | 2146 | adenine phosphoribosyltransferase (EC 2.4.2.7) - like protein | 0 | At4g22570 | 0.46 |
| 2147 | 2148 | unknown protein | 0 | At3g15950 | 0.37 |
| 2149 | 2150 | putative glutathione peroxidase | 7E-12 | At2g25080 | 0.46 |
| 2151 | 2152 | putative protein | 0 | At5g23060 | 0.63 |
| 2153 | 2154 | pectate lyase 1-like protein | 0 | At1g67750 | 0.42 |
| 2155 | 2156 | putative triosephosphate isomerase | 9E-61 | At2g21170 | 0.66 |
| 2157 | 2158 | carbonate dehydratase - like protein | 0 | At4g33580 | 0.72 |
| 2159 | 2160 | putative protein | 0 | At5g37300 | 0.56 |
| 2161 | 2162 | putative protein | 1E-143 | At3g60080 | 0.77 |
| 2163 | 2164 | cystatin (emb\|CAA03929.1) | 2E-83 | At5g12140 | 0.74 |
| 2165 | 2166 | putative cytochrome b5 | 0 | At2g46650 | 0.46 |
| 2167 | 2168 | putaive DNA-binding protein | 2E-08 | At4g31550 | 0.63 |
| 2169 | 2170 | hypothetical protein | 1E-143 | At3g21050 | 0.5 |
| 2171 | 2172 | putative beta-hydroxyacyl-ACP dehydratase | 0 | At2g22230 | 0.59 |
| 2173 | 2174 | 2-oxoglutarate/malate translocator | 0 | At5g64290 | 0.77 |
| 2175 | 2176 | hypothetical protein | 1E-123 | At3g27050 | 0.49 |
| 2177 | 2178 | putative alcohol dehydrogenase | 9E-64 | At2g37770 | 0.64 |
| 2179 | 2180 | hypothetical protein | 1E-107 | At1g18730 | 0.67 |
| 2181 | 2182 | putative pectinacetylesterase | 0 | At4g19420 | 0.71 |
| 2183 | 2184 | similar to ADP-ribosylation factor gb\|AAD17207; similar to ESTs | 2E-80 | At1g10630 | 0.67 |
| 2185 | 2186 | hypothetical protein | 0 | At1g04420 | 0.67 |
| 2187 | 2188 | putative protein | 0 | At4g26710 | 0.62 |
| 2189 | 2190 | putative protein | 0 | At4g34630 | 0.72 |
| 2191 | 2192 | latex protein | 0 | At1g70890 | 0.29 |
| 2193 | 2194 | RCc3- like protein | 0 | At4g22490 | 0.57 |
| 2195 | 2196 | hypothetical protein | 5E-53 | At1g20450 | 0.49 |
| 2197 | 2198 | glucosyltransferase-like protein | 3E-31 | At5g22740 | 0.65 |
| 2199 | 2200 | glutathione S-transferase | 0 | At2g29450 | 0.52 |
| 2201 | 2202 | putative protein | 0 | At3g44450 | 0.59 |
| 2203 | 2204 | cysteine synthase | 0 | At5g28020 | 0.6 |
| 2205 | 2206 | ATP synthase | 0 | At4g04640 | 0.57 |
| 2207 | 2208 | 40S ribosomal protein S14 | 1E-25 | At2g36160 | 0.67 |
| 2209 | 2210 | putative protein | 0 | At4g19100 | 0.76 |
| 2211 | 2212 | K Efflux antiporter KEA1 | 0 | At1g01790 | 0.65 |
| 2213 | 2214 | hypothetical protein | 1E-169 | At2g42980 | 0.66 |
| 2215 | 2216 | cytochrome P450 like protein | 1E-01 | At4g36380 | 0.48 |
| 2217 | 2218 | unknown protein | 8E-64 | At2g01520 | 0.23 |
| 2219 | 2220 | hypothetical protein | 1E-157 | At1g07130 | 0.66 |
| 2221 | 2222 | putative protein | 5E-04 | At5g09620 | 0.62 |
| 2223 | 2224 | unknown protein | 0 | At1g08470 | 0.66 |
| 2225 | 2226 | putative protein | 6E-37 | At3g54600 | 0.7 |
| 2227 | 2228 | DnaJ - like protein | 1E-68 | At4g39960 | 0.52 |
| 2229 | 2230 | putative protein phosphatase 2C | 1E-161 | At1g78200 | 0.72 |
| 2231 | 2232 | biotin synthase (Bio B) | 0 | At2g43360 | 0.67 |
| 2233 | 2234 | unknown protein | 3E-69 | At3g17510 | 0.55 |
| 2235 | 2236 | high mobility group protein 2-like | 1E-107 | At3g51880 | 0.66 |
| 2237 | 2238 | putative proline-rich protein | 0 | At2g21140 | 0.57 |
| 2239 | 2240 | cyclin delta-3 | 0 | At4g34160 | 0.74 |
| 2241 | 2242 | serine carboxypeptidase II - like protein | 0 | At4g30810 | 0.77 |
| 2243 | 2244 | unknown protein | 0 | At1g67330 | 0.7 |
| 2245 | 2246 | putative protein | 7E-93 | At3g56010 | 0.7 |

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|---|---|---|---|---|---|
| 2247 | 2248 | GTP-binding protein LepA homolog | 0 | At5g08650 | 0.76 |
| 2249 | 2250 | unknown protein | 0 | At3g10420 | 0.42 |
| 2251 | 2252 | putative protein | 0 | At3g51510 | 0.58 |
| 2253 | 2254 | putative protein | 0 | At3g45870 | 0.73 |
| 2255 | 2256 | putative enolase | 0 | At1g74030 | 0.65 |
| 2257 | 2258 | putative protein | 3E-05 | At5g11680 | 0.71 |
| 2259 | 2260 | putative protein | 0 | At5g26280 | 0.58 |
| 2261 | 2262 | O-methyltransferase, putative | 0 | At1g21100 | 0.63 |
| 2263 | 2264 | beta-1,3-glucanase class I precursor | 0 | At4g16260 | 0.51 |
| 2265 | 2266 | protein phosphatase 2C (PP2C) | 2E-27 | At3g11410 | 0.67 |
| 2267 | 2268 | root cap protein 2-like protein | 1E-174 | At5g54370 | 0.75 |
| 2269 | 2270 | putative adenosine phosphosulfate kinase | 0 | At2g14750 | 0.47 |
| 2271 | 2272 | putative protein | 0 | At4g30010 | 0.73 |
| 2273 | 2274 | putative uroporphyrinogen decarboxylase | 2 E-9 | At2g40490 | 0.75 |
| 2275 | 2276 | putative protein | 1E-151 | At3g57400 | 0.71 |
| 2277 | 2278 | branched-chain amino acid aminotransferase, putative | 1E-56 | At3g19710 | 0.3 |
| 2279 | 2280 | copia-like retroelement pol polyprotein | 0 | At2g19830 | 0.72 |
| 2281 | 2282 | neoxanthin cleavage enzyme-like protein | 0 | At4g19170 | 0.38 |
| 2283 | 2284 | hypothetical protein | 0 | At1g31860 | 0.7 |
| 2285 | 2286 | unknown protein | 0 | At2g26570 | 0.61 |
| 2287 | 2288 | asparagine synthetase ASN3 | 0 | At5g10240 | 0.72 |
| 2289 | 2290 | hypothetical protein | 1E-80 | At1g64770 | 0.56 |
| 2291 | 2292 | expansin S2 precursor, putative | 1E-114 | At1g20190 | 0.51 |
| 2293 | 2294 | 5'-adenylylsulfate reductase | 0 | At4g04610 | 0.43 |
| 2295 | 2296 | putative protein | 9E-02 | At3g59680 | 0.71 |
| 2297 | 2298 | putative MYB family transcription factor | 4E-31 | At2g37630 | 0.73 |
| 2299 | 2300 | Putative protein kinase | 3E-23 | At1g51850 | 0.6 |
| 2301 | 2302 | putative protein | 0 | At5g15910 | 0.76 |
| 2303 | 2304 | AALP protein | 0 | At5g60360 | 0.63 |
| 2305 | 2306 | putative galactinol synthase | 0 | At2g47180 | 0.69 |
| 2307 | 2308 | cyanohydrin lyase like protein | 0 | At4g16690 | 0.56 |
| 2309 | 2310 | putative protein | 0 | At5g03880 | 0.57 |
| 2311 | 2312 | putative glucosyltransferase | 0 | At2g30150 | 0.73 |
| 2313 | 2314 | cysteine endopeptidase precursor - like protein | 0 | At3g48350 | 0.65 |
| 2315 | 2316 | unknown protein | 1E-122 | At3g07700 | 0.7 |
| 2317 | 2318 | putative peroxiredoxin | 2E-86 | At3g26060 | 0.76 |
| 2319 | 2320 | MAPKK | 0 | At1g73500 | 0.58 |
| 2321 | 2322 | hypothetical protein | 7E-74 | At1g64780 | 0.52 |
| 2323 | 2324 | UDP glucose:flavonoid 3-o-glucosyltransferase, putative | 2E-90 | At1g30530 | 0.59 |
| 2325 | 2326 | hypothetical protein | 0 | At4g02800 | 0.55 |
| 2327 | 2328 | oxidoreductase -like protein | 0 | At3g55290 | 0.65 |
| 2329 | 2330 | hypothetical protein | 0 | At1g50670 | 0.73 |
| 2331 | 2332 | carnitine/acylcarnitine translocase-like protein | 0 | At5g46800 | 0.58 |
| 2333 | 2334 | MATH protein | 1E-169 | At4g00780 | 0.57 |
| 2335 | 2336 | unknown protein | 0 | At1g22630 | 0.76 |
| 2337 | 2338 | cytochrome P450-like protein | 0 | At4g37330 | 0.72 |
| 2339 | 2340 | putative endo-1,4-beta glucanase | 8E-36 | At4g02290 | 0.62 |
| 2341 | 2342 | hevein-like protein precursor | 0E+00 | At3g04720 | 0.75 |
| 2343 | 2344 | leucine zipper-containing protein AT103 | 1E-139 | At3g56940 | 0.63 |
| 2345 | 2346 | delta-1-pyrroline-5-carboxylate synthetase | 0 | At3g55610 | 0.69 |
| 2347 | 2348 | remorin | 0 | At2g45820 | 0.76 |
| 2349 | 2350 | putative protein | 0 | At5g22460 | 0.48 |

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|---|---|---|---|---|---|
| 2351 | 2352 | putative lectin | 0 | At3g16530 | 0.43 |
| 2353 | 2354 | putative protein | 9E-29 | At5g26260 | 0.52 |
| 2355 | 2356 | peptidylprolyl isomerase ROC4 | 0 | At3g62030 | 0.61 |
| 2357 | 2358 | O-methyltransferase, putative | 0 | At1g21130 | 0.63 |
| 2359 | 2360 | putative zinc finger protein | 0 | At4g38960 | 0.72 |
| 2361 | 2362 | putative hydroxyproline-rich glycoprotein | 1E-173 | At1g13930 | 0.58 |
| 2363 | 2364 | putative protein 1 photosystem II oxygen-evolving complex | 0 | At3g50820 | 0.65 |
| 2365 | 2366 | hypothetical protein | 0 | At1g66700 | 0.63 |
| 2367 | 2368 | unknown protein | 0 | At1g52870 | 0.43 |
| 2369 | 2370 | heat shock protein 90 | 0 | At5g56010 | 0.75 |
| 2371 | 2372 | Overlap with bases 87,142-90,425 of 'IGF' BAC clone F9K20, accession | 1E-115 | At1g78570 | 0.63 |
| 2373 | 2374 | phosphoglycerate kinase, putative | 1E-120 | At3g12780 | 0.73 |
| 2375 | 2376 | putative lectin | 1E-25 | At3g16400 | 0.4 |
| 2377 | 2378 | profilin 2 | 0 | At4g29350 | 0.77 |
| 2379 | 2380 | HSP associated protein like | 5E-16 | At4g22670 | 0.75 |
| 2381 | 2382 | putative cell division control protein, cdc2 kinase | 1E-75 | At1g20930 | 0.72 |
| 2383 | 2384 | putative protein | 1E-107 | At5g08050 | 0.65 |
| 2385 | 2386 | ribosomal protein S27 | 0 | At5g47930 | 0.77 |
| 2387 | 2388 | vacuolar H+-transporting ATPase 16K chain | 0 | At4g34720 | 0.76 |
| 2389 | 2390 | expansin At-EXP5 | 3E-82 | At3g29030 | 0.52 |
| 2391 | 2392 | similar to cold acclimation protein WCOR413 [Triticum aestivum] | 0 | At2g15970 | 0.74 |
| 2393 | 2394 | chloroplast membrane protein (ALBINO3) | 1E-159 | At2g28800 | 0.72 |
| 2395 | 2396 | putative thioredoxin | 1E-102 | At1g08570 | 0.55 |
| 2397 | 2398 | unknown protein | 0 | At1g08380 | 0.65 |
| 2399 | 2400 | hypothetical protein | 0 | At1g07180 | 0.53 |
| 2401 | 2402 | putative flavonol sulfotransferase | 0 | At1g74090 | 0.69 |
| 2403 | 2404 | possible apospory-associated like protein | 0 | At4g25900 | 0.71 |
| 2405 | 2406 | glycolate oxidase, putative | 0 | At3g14420 | 0.71 |
| 2407 | 2408 | putative peroxidase ATP2a | 0 | At2g37130 | 0.75 |
| 2409 | 2410 | putative protein | 1E-154 | At4g21860 | 0.75 |
| 2411 | 2412 | hydroxypyruvate reductase (HPR) | 0 | At1g68010 | 0.74 |
| 2413 | 2414 | photosystem I reaction centre subunit psaN precursor (PSI-N) | 0 | At5g64040 | 0.49 |
| 2415 | 2416 | plastid ribosomal protein S6, putative | 0 | At1g64510 | 0.6 |
| 2417 | 2418 | methylenetetrahydrofolate reductase MTHFR1 | 0 | At3g59970 | 0.72 |
| 2419 | 2420 | putative photosystem I reaction center subunit II precursor | 0 | At1g03130 | 0.55 |
| 2421 | 2422 | unknown protein | 0 | At3g10940 | 0.64 |
| 2423 | 2424 | fumarate hydratase | 0 | At5g50950 | 0.43 |
| 2425 | 2426 | Lil3 protein | 0 | At5g47110 | 0.73 |
| 2427 | 2428 | homeobox gene ATH1 | 0 | At4g32980 | 0.76 |
| 2429 | 2430 | putative lectin | 3E-20 | At3g16390 | 0.43 |
| 2431 | 2432 | COP1-interacting protein 7 (CIP7) | 1E-07 | At4g27430 | 0.67 |
| 2433 | 2434 | putative acyl-CoA synthetase | 0 | At2g47240 | 0.51 |
| 2435 | 2436 | unknown protein | 0 | At2g01590 | 0.68 |
| 2437 | 2438 | hydroxymethyltransferase | 0 | At4g13930 | 0.72 |
| 2439 | 2440 | hypothetical protein | 1E-164 | At1g69490 | 0.27 |
| 2441 | 2442 | SNF1 related protein kinase (ATSRPK1) | 1E-170 | At3g23000 | 0.49 |
| 2443 | 2444 | mevalonate diphosphate decarboxylase | 6E-68 | At2g38700 | 0.71 |
| 2445 | 2446 | putative flavonol sulfotransferase | 0 | At1g74090 | 0.69 |
| 2447 | 2448 | protein phosphatase 2C (AtP2C-HA) | 0 | At1g72770 | 0.59 |
| 2449 | 2450 | cinnamoyl-CoA reductase - like protein | 0 | At4g30470 | 0.72 |
| 2451 | 2452 | O-methyltransferase - like protein | 0 | At4g35160 | 0.5 |

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|---|---|---|---|---|---|
| 2453 | 2454 | pyruvate dehydrogenase E1 alpha subunit | 0 | At1g01090 | 0.77 |
| 2455 | 2456 | putative chlorophyll A-B binding protein | 0 | At3g27690 | 0.49 |
| 2457 | 2458 | putative UDP-N-acetylglucosamine pyrophosphorylase | 0 | At2g35020 | 0.69 |
| 2459 | 2460 | putative protein | 1E-121 | At4g05590 | 0.75 |
| 2461 | 2462 | Ca2+-dependent membrane-binding protein annexin | 0 | At1g35720 | 0.41 |
| 2463 | 2464 | hypothetical protein | 0 | At2g35760 | 0.51 |
| 2465 | 2466 | hypothetical protein | 2E-15 | At1g18840 | 0.71 |
| 2467 | 2468 | hypothetical protein | 0 | At1g51140 | 0.53 |
| 2469 | 2470 | aromatic amino-acid decarboxylase - like protein | 0 | At4g28680 | 0.73 |
| 2471 | 2472 | unknown protein | 3E-72 | At2g35830 | 0.49 |
| 2473 | 2474 | hypothetical protein | 0 | At1g78690 | 0.66 |
| 2475 | 2476 | putative elongation factor P (EF-P) | 0 | At3g08740 | 0.74 |
| 2477 | 2478 | unknown protein | 0 | At1g22750 | 0.76 |
| 2479 | 2480 | putative protein | 0 | At3g63160 | 0.45 |
| 2481 | 2482 | unknown protein | 1E-150 | At3g26510 | 0.55 |
| 2483 | 2484 | aldo/keto reductase-like protein | 0 | At5g53580 | 0.69 |
| 2485 | 2486 | glycine decarboxylase complex H-protein | 0 | At2g35370 | 0.53 |
| 2487 | 2488 | thioredoxin (clone GIF1) (pir\|\|S58118) | 3E-14 | At5g42980 | 0.53 |
| 2489 | 2490 | putative protein | 1E-93 | At4g28020 | 0.52 |
| 2491 | 2492 | hypothetical protein | 0 | At1g18870 | 0.71 |
| 2493 | 2494 | vegetative storage protein Vsp2 | 0 | At5g24770 | 0.43 |
| 2495 | 2496 | putative protein | 3E-75 | At4g17560 | 0.66 |
| 2497 | 2498 | NBD-like protein (gb\|AAD20643.1) | 0E+00 | At5g44110 | 0.58 |
| 2499 | 2500 | photosystem I subunit V precursor, putative | 1E-119 | At1g55670 | 0.56 |
| 2501 | 2502 | putative thaumatin | 2E-36 | At2g28790 | 0.64 |
| 2503 | 2504 | hyoscyamine 6-dioxygenase hydroxylase, putative | 0 | At1g35190 | 0.71 |
| 2505 | 2506 | H-protein promoter binding factor-like protein | 0 | At5g62430 | 0.51 |
| 2507 | 2508 | putative protein | 0 | At4g04840 | 0.52 |
| 2509 | 2510 | endo-xyloglucan transferase - like protein | 0 | At4g37800 | 0.68 |
| 2511 | 2512 | vitamine c-2 | 0 | At4g26850 | 0.33 |
| 2513 | 2514 | hypothetical protein | 0 | At3g12340 | 0.69 |
| 2515 | 2516 | putative acetone-cyanohydrin lyase | 0 | At2g23610 | 0.68 |
| 2517 | 2518 | putative transcription factor | 0 | At1g71030 | 0.36 |
| 2519 | 2520 | hypothetical protein | 1E-128 | At1g19000 | 0.74 |
| 2521 | 2522 | putative xyloglucan endo-transglycosylase | 7E-27 | At2g36870 | 0.4 |
| 2523 | 2524 | hypothetical protein | 3E-51 | At1g58080 | 0.77 |
| 2525 | 2526 | putative protein | 1E-167 | At5g36800 | 0.65 |
| 2527 | 2528 | putative protein | 1E-157 | At4g30530 | 0.65 |
| 2529 | 2530 | cinnamyl-alcohol dehydrogenase ELI3-1 | 0 | At4g37980 | 0.54 |
| 2531 | 2532 | putative CONSTANS-like B-box zinc finger protein | 0 | At2g47890 | 0.72 |
| 2533 | 2534 | unknown protein | 1E-123 | At1g53480 | 0.6 |
| 2535 | 2536 | protein phosphatase 2C-like protein | 2E-55 | At4g28400 | 0.72 |
| 2537 | 2538 | putative protein | 0 | At5g60680 | 0.57 |
| 2539 | 2540 | farnesyl-pyrophosphate synthetase FPS2 | 0 | At4g17190 | 0.76 |
| 2541 | 2542 | soluble inorganic pyrophosphatase, putative | 0 | At1g01050 | 0.59 |
| 2543 | 2544 | putative nematode-resistance protein | 1E-117 | At2g40000 | 0.34 |
| 2545 | 2546 | putative AP2 domain transcription factor | 0 | At2g23340 | 0.74 |
| 2547 | 2548 | putative myo-inositol monophosphatase | 3E-17 | At3g02870 | 0.6 |
| 2549 | 2550 | putative isoamylase | 0 | At1g03310 | 0.74 |
| 2551 | 2552 | phosphate transporter (AtPT2) | 0 | At2g38940 | 0.76 |
| 2553 | 2554 | putative disease resistance response protein | 0 | At4g11190 | 0.68 |
| 2555 | 2556 | unknown protein | 0 | At2g45600 | 0.55 |

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| 2557 | 2558 | peroxidase ATP13a | 0 | At5g17820 | 0.7 |
| 2559 | 2560 | unknown protein | 0 | At1g26920 | 0.74 |
| 2561 | 2562 | putative mitochondrial carrier protein | 0 | At2g47490 | 0.69 |
| 2563 | 2564 | actin depolymerizing factor 3 - like protein | 1E-136 | At5g59880 | 0.64 |
| 2565 | 2566 | putative protein transport protein SEC23 | 1E-149 | At2g21630 | 0.73 |
| 2567 | 2568 | unknown protein | 2E-30 | At2g44310 | 0.74 |
| 2569 | 2570 | putative protein | 0 | At4g21570 | 0.69 |
| 2571 | 2572 | putative steroid binding protein | 0 | At2g24940 | 0.57 |
| 2573 | 2574 | putative lipid transfer protein | 0 | At2g15050 | 0.49 |
| 2575 | 2576 | hypothetical protein | 0 | At4g15510 | 0.75 |
| 2577 | 2578 | unknown protein | 3E-47 | At3g25690 | 0.56 |
| 2579 | 2580 | 40S ribosomal protein S19 - like | 0 | At5g28060 | 0.73 |
| 2581 | 2582 | putative auxin-regulated protein | 0 | At2g21210 | 0.56 |
| 2583 | 2584 | unknown protein | 0 | At1g19350 | 0.71 |
| 2585 | 2586 | unknown protein | 1E-136 | At1g07700 | 0.71 |
| 2587 | 2588 | 50S ribosomal protein L27 | 0E+00 | At5g40950 | 0.7 |
| 2589 | 2590 | unknown protein | 1E-105 | At2g46540 | 0.69 |
| 2591 | 2592 | ATP-sulfurylase | 0 | At4g14680 | 0.72 |
| 2593 | 2594 | hypothetical protein | 1E-107 | At3g18890 | 0.64 |
| 2595 | 2596 | putative protein | 0 | At3g59780 | 0.62 |
| 2597 | 2598 | cytochrome P450 monooxygenase - like protein | 0 | At4g37410 | 0.56 |
| 2599 | 2600 | hypothetical protein | 2E-86 | At1g61890 | 0.36 |
| 2601 | 2602 | ubiquitin-conjugating enzyme, putative | 0 | At3g20060 | 0.66 |
| 2603 | 2604 | hypothetical protein | 0 | At1g20810 | 0.74 |
| 2605 | 2606 | hypothetical protein | 0 | At2g15020 | 0.45 |
| 2607 | 2608 | unknown protein | 0 | At1g55480 | 0.52 |
| 2609 | 2610 | UDP glucose:flavonoid 3-o-glucosyltransferase -like protein | 0 | At5g17050 | 0.56 |
| 2611 | 2612 | hypothetical protein | 0 | At3g23670 | 0.69 |
| 2613 | 2614 | putative protein | 0 | At4g34920 | 0.69 |
| 2615 | 2616 | unknown protein | 1E-100 | At2g36630 | 0.71 |
| 2617 | 2618 | unknown protein | 6E-94 | At1g56580 | 0.63 |
| 2619 | 2620 | HSR201 like protein | 0 | At4g15390 | 0.75 |
| 2621 | 2622 | heme oxygenase 1 (HO1) | 0 | At2g26670 | 0.74 |
| 2623 | 2624 | putative beta-glucosidase | 0 | At4g27820 | 0.46 |
| 2625 | 2626 | unknown protein | 1E-122 | At1g68440 | 0.45 |
| 2627 | 2628 | predicted protein | 0 | At4g22820 | 0.54 |
| 2629 | 2630 | putative kinesin heavy chain | 0 | At2g22610 | 0.72 |
| 2631 | 2632 | putative protein | 0 | At4g27860 | 0.61 |
| 2633 | 2634 | unknown protein | 0 | At2g37240 | 0.76 |
| 2635 | 2636 | unknown protein | 0 | At1g30070 | 0.76 |
| 2637 | 2638 | WD-repeat protein -like protein | 0 | At4g33270 | 0.57 |
| 2639 | 2640 | unknown protein | 0 | At1g32220 | 0.6 |
| 2641 | 2642 | hypothetical protein | 0 | At4g22920 | 0.73 |
| 2643 | 2644 | putative amino acid transporter protein | 0 | At3g11900 | 0.67 |
| 2645 | 2646 | endo-beta-1,4-glucanase, putative | 0 | At1g64390 | 0.5 |
| 2647 | 2648 | hypothetical protein | 0 | At1g18060 | 0.7 |
| 2649 | 2650 | hypothetical protein | 1E-114 | At4g39820 | 0.7 |
| 2651 | 2652 | putative protein | 1E-62 | At5g27290 | 0.6 |
| 2653 | 2654 | putative protein | 1E-133 | At3g48200 | 0.46 |
| 2655 | 2656 | hypothetical protein | 1E-173 | At1g64500 | 0.51 |
| 2657 | 2658 | putative ribonuclease, RNS2 | 0 | At2g39780 | 0.6 |
| 2659 | 2660 | thioredoxin f1 | 0 | At3g02730 | 0.59 |

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|---|---|---|---|---|---|
| 2661 | 2662 | unknown protein | 0 | At2g20670 | 0.67 |
| 2663 | 2664 | cytochrome P450-like protein | 0 | At5g48000 | 0.45 |
| 2665 | 2666 | subtilisin proteinase - like | 1E-105 | At4g21650 | 0.31 |
| 2667 | 2668 | photoassimilate-responsive protein PAR-1b -like protein | 0E+00 | At3g54040 | 0.76 |
| 2669 | 2670 | putative dTDP-glucose 4-6-dehydratase | 0 | At2g27860 | 0.45 |
| 2671 | 2672 | hypothetical protein | 0 | At1g51700 | 0.43 |
| 2673 | 2674 | early light-induced protein | 0 | At3g22840 | 0.65 |
| 2675 | 2676 | hypothetical protein | 0 | At1g32060 | 0.42 |
| 2677 | 2678 | unknown protein | 0 | At2g34860 | 0.69 |
| 2679 | 2680 | peroxidase ATP3a (emb\|CAA67340.1) | 4E-10 | At5g64100 | 0.49 |
| 2681 | 2682 | putative protein | 0 | At5g06770 | 0.67 |
| 2683 | 2684 | hypothetical protein | 0 | At2g16860 | 0.57 |
| 2685 | 2686 | annexin | 0 | At5g65020 | 0.61 |
| 2687 | 2688 | thioredoxin, putative | 0 | At1g50320 | 0.63 |
| 2689 | 2690 | putative protein | 0 | At5g17360 | 0.66 |
| 2691 | 2692 | nucleoside diphosphate kinase 3 (ndpk3) | 0 | At4g11010 | 0.76 |
| 2693 | 2694 | unknown protein | 0 | At5g62550 | 0.64 |
| 2695 | 2696 | putative protein | 0 | At4g12000 | 0.62 |
| 2697 | 2698 | cell division protease FtsH, putative | 0 | At1g06430 | 0.65 |
| 2699 | 2700 | unknown protein | 0 | At1g74880 | 0.41 |
| 2701 | 2702 | putative protein | 0 | At5g56540 | 0.61 |
| 2703 | 2704 | unknown protein | 0 | At1g68780 | 0.61 |
| 2705 | 2706 | mipC protein - like (aquaporin) | 0 | At5g60660 | 0.64 |
| 2707 | 2708 | Oxygen-evolving enhancer protein 3 precursor - like protein | 0 | At4g05180 | 0.64 |
| 2709 | 2710 | cytochrome P450, putative | 0 | At3g26180 | 0.74 |
| 2711 | 2712 | putative protein | 1E-126 | At5g22210 | 0.74 |
| 2741 | 2742 | unknown protein | | At1g45200 | 3.91 |
| 2743 | 2744 | unknown protein, putative protease inhibitor | | At5g43580 | 2.58 |
| 2745 | 2746 | putative protein | | At5g03540 | 2.21 |
| 2747 | 2748 | putative WD repeat protein | | At3g15880 | 2.38 |
| 2749 | 2750 | putative protease inhibitor Dr4 | | At1g73330 | 10.30 |
| 2751 | 2752 | putative auxin regulated protein | | At2g46690 | 2.86 |
| 2753 | 2754 | translation initiation factor like protein | | At5g54940 | 2.15 |
| 2755 | | pseudogene | | At2g04110 | 2.07 |

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## CLAIMS

1. A method to alter one or more plant characteristics, said method comprising modifying, in a plant, expression of one or more nucleic acids and/or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one of SEQ ID NO 1 to 2755, and wherein said one or more plant characteristics are altered relative to corresponding wild type plants.

2. A method according to claim 1, wherein said altered plant characteristic is selected from any one or more of the following: altered development, altered growth, increased yield and/or biomass, enhanced survival capacity, enhanced stress tolerance, altered plant architecture, altered plant physiology, altered plant biochemistry, altered metabolism, altered DNA synthesis, altered DNA modification, altered endoreduplication, altered cell cycle, altered cell wall biogenesis, altered transcription regulation, altered signal transduction, altered storage lipid mobilization and/or altered photosynthesis, each relative to corresponding wild type plants.

3. A method according to claim 2, wherein said altered metabolism comprises altered nitrogen and/or altered carbon metabolism.

4. A method according to claim 2, wherein said increased yield and/or biomass, comprises increased seed yield.

5. A recombinant nucleic acid comprising:

(a) one or more nucleic acid sequences essentially similar to any one of SEQ ID NO 1 to 2755 or the complement strand thereof; optionally operably linked to

(b) a regulatory sequence, and optionally operably linked to

(c) a transcription termination sequence

6. A recombinant nucleic acid according to claim 5, wherein said regulatory sequence is a plant-expressible promoter.

7. A recombinant nucleic acid according to claims 6, wherein said plant-expressible promoter is any one of the promoters listed in Table I, II, III or IV.

8. A method for making a transgenic plant or plant cell having one or more altered plant characteristics when compared to the corresponding wild-type characteristics, said method comprising introduction of a recombinant nucleic acid according to claim 5, 6 or 7 into said plant or plant cell.

9. A method according to claim 8, wherein said recombinant nucleic acid is stably integrated into the genome of said plant .

10. A method according to any of claims 1 to 4 or 8 or 9, comprising overexpression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755.

11. A method according to any of claims 1 to 4 or 8 or 9, comprising downregulation of expression of one or more nucleic acids essentially similar to any one of SEQ ID NO 1 to 2755.

12. A transgenic plant having one or more altered characteristics when compared to the corresponding wild-type plant, characterized in that said plant has modified expression of one or more nucleic acids and/or modified level and/or activity of one or more proteins, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

13. A transgenic plant obtainable by a method according to any of claims 1 to 4 or 8 to 11.

14. A transgenic plant comprising an isolated nucleic acid and/or protein sequence essentially similar to any one of SEQ ID NO 1 to 2755.

15. An ancestor, progeny, or any plant part, particularly a harvestable part, of a transgenic plant of claim 12 or 14.

16. A host cell having one or more altered characteristics when compared to the corresponding wild-type host cell, characterized in that said host cell has modified expression of one or more nucleic acids and/or modified level and/or activity of one or more proteins, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

17. Use of a nucleic acid sequence or protein essentially similar to any one of SEQ ID NO 1 to 2755, for altering one or more plant characteristics.

18. A method for the production of plants with one or more altered characteristics when

compared to the corresponding wild-type plants, which method comprises the use of a nucleic acid sequence essentially similar to any of SEQ ID NO 1 to 2755 in marker assisted breeding.

19. A method for the production of plants with one or more altered characteristics when compared to the corresponding wild-type plants, which method comprises the use of a nucleic acid sequence essentially similar to any of SEQ ID NO 1 to 2755 in conventional breeding.

20. A plant obtainable by the methods according to claim 18 or 20.

21. Use of a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a growth regulator.

22. A growth regulating composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

23. A method for the production of a growth regulator, comprising the production of a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

24. Method for the production of enzymes and/or pharmaceuticals, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

25. Use of plants according to claim 11 to 14, for the production of enzymes and/or pharmaceuticals.

26. Enzymes and pharmaceuticals produced according to the method of claim 24.

27. Use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755, as a therapeutic agent, a diagnostic means, a kit or plant effective agent.

28. A therapeutic composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

29. A diagnostic composition comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

30. A kit comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

31. A plant effective agent comprising a nucleic acid and/or a protein essentially similar to any one of SEQ ID NO 1 to 2755.

32. Method for the manufacturing of a therapeutic composition, a diagnostic composition, a kit or a plant effective agent, comprising the production of a sequence essentially similar to any one of SEQ ID NO 1 to 2755.

33. A food product derived from a plant or host cell according to any one of claim 12 to 16.

34. Use of a food product according to claim 33 in animal feed or food.

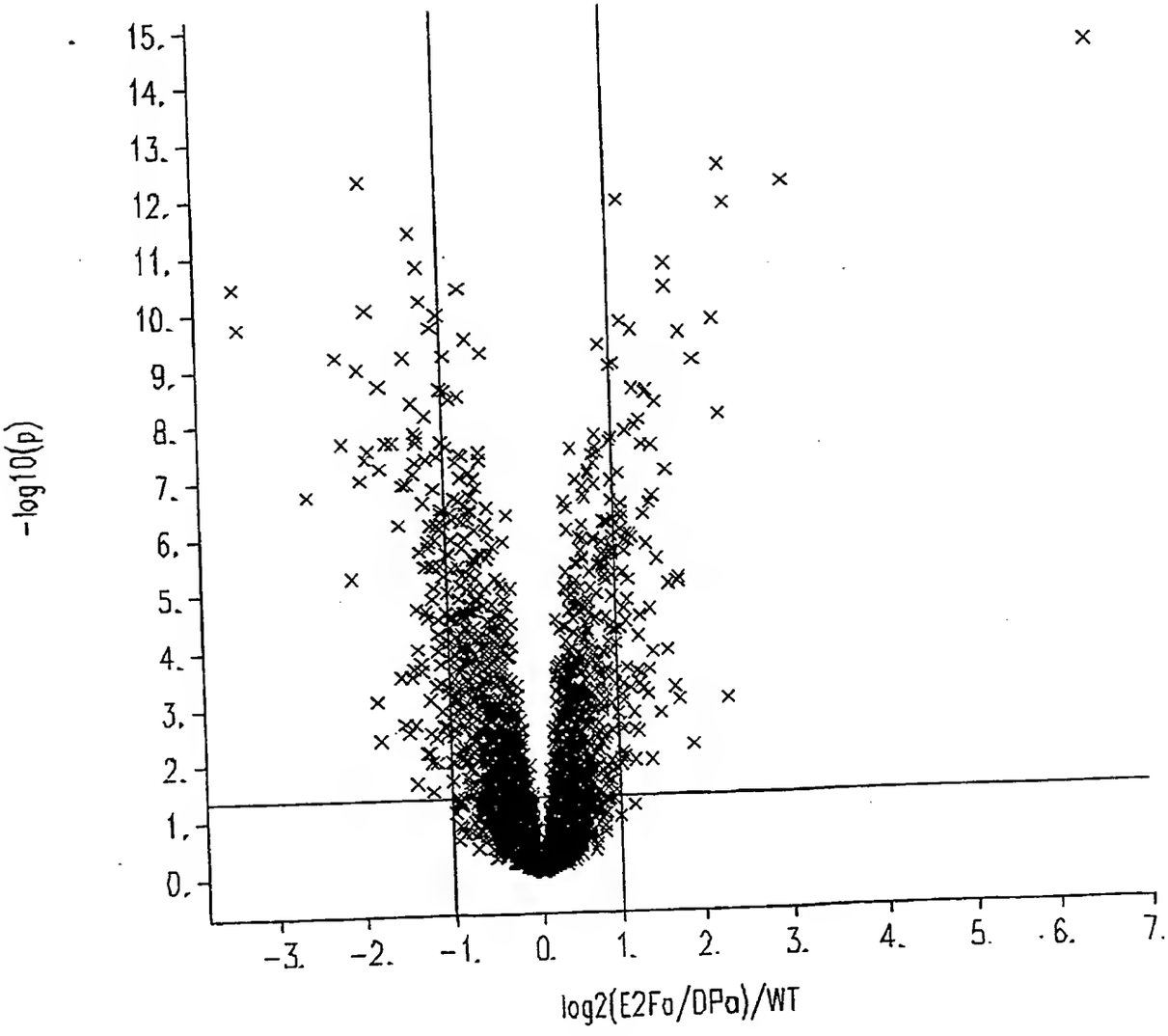
35. Method for the production of a food or feed product, which method comprises modifying expression of a nucleic acid, and/or modifying level and/or activity of a protein, said nucleic acid and/or protein being essentially similar to any one of SEQ ID NO 1 to 2755.

36. Use of a nucleic acid or a protein essentially similar to any one of SEQ ID NO 1 to 2755 as a positive or negative selectable marker during transformation of cells or tissues or during cell procedures.

37. Use according to claim 36, wherein said cell is derived from a plant, animal, bacterium, fungus, yeast, insect, algae.

38. An isolated nucleic acid comprising one or more of the regulatory elements upstream of the startcodon of any of the nucleic acids represented by SEQ ID NO 1 to 2755.

39. An isolated nucleic acid according to claim 38, wherein said regulatory element is the natural promoter of any one of said nucleic acids represented by SEQ ID NO 1 to 2755.


15.
14.
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9.
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-log10(p)
-3.
-2.
-1.
0.
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7.
log2(E2Fa/DPa)/WT

FIGURE 1

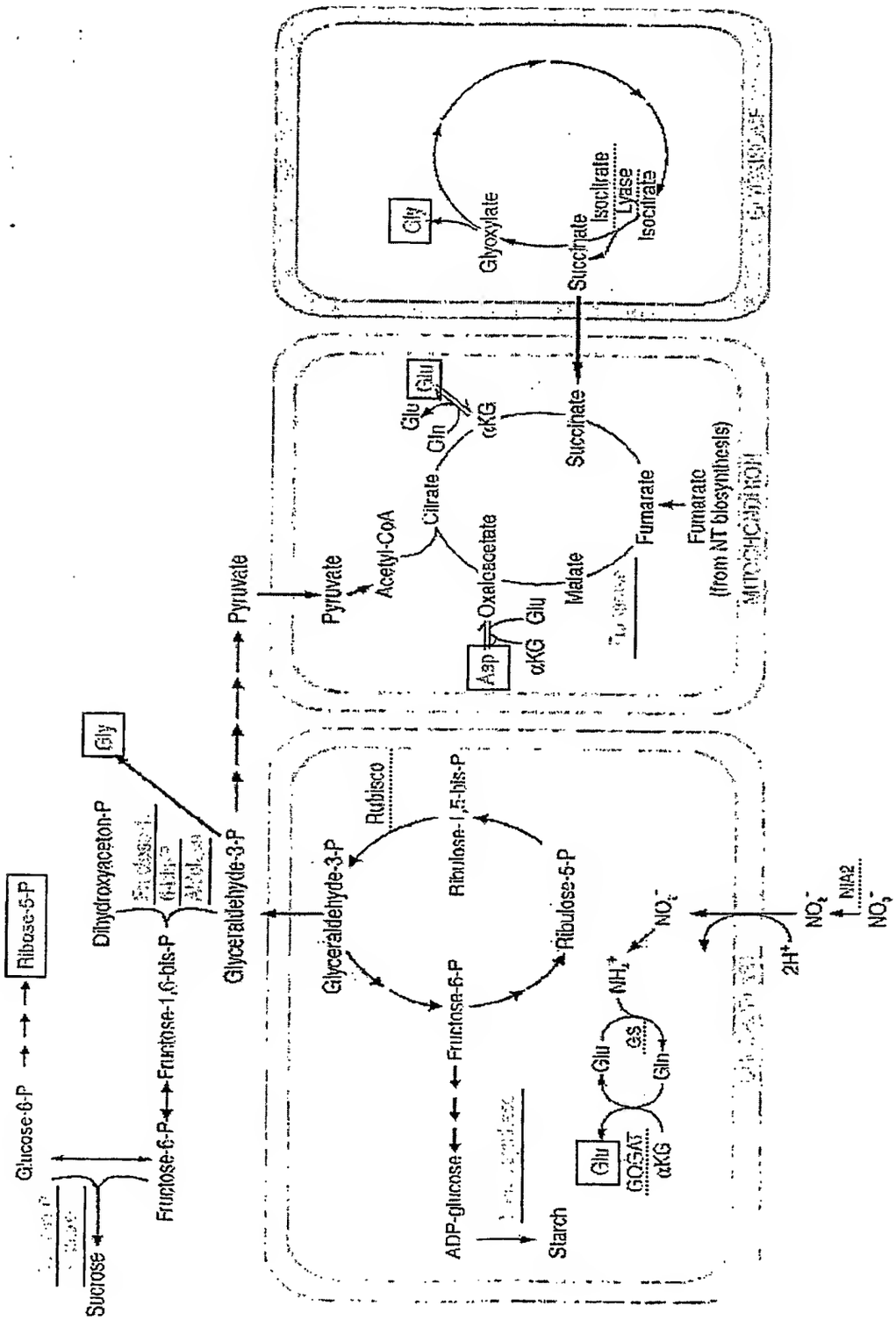
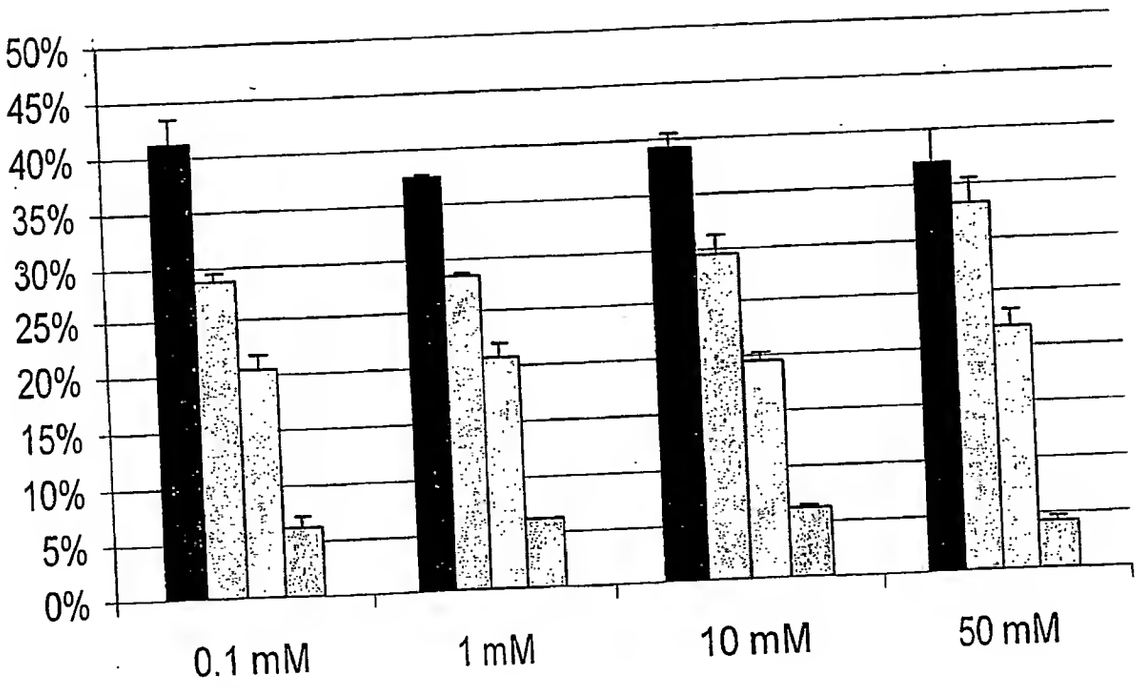

Sucrose
Glucose-6-P
Ribose-5-P
Fructose-6-P
Fructose-1,6-bis-P
Dihydroxyaceton-P
Gly
Glyceraldehyde-3-P
Pyruvate
Rubisco
Ribulose-1,5-bis-P
Ribulose-5-P
Glyceraldehyde-3-P
Fructose-6-P
ADP-glucose
Starch
Pyruvate
Acetyl-CoA
Citrate
Oxalacetate
Malate
Fumarate
Fumarate (from NT biosynthesis)
Glu
Gln
αKG
Asp
Succinate
Glyoxylate
Isocitrate Lyase
Isocitrate
Gly
Glu
Gln
GS
GOGAT
αKG
$NH_4^+$
$NO_2^-$
$2H^+$
$NO_2^-$
$NH_4^+$
$NO_3^-$

FIGURE 2

A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0.1 mM
1 mM
10 mM
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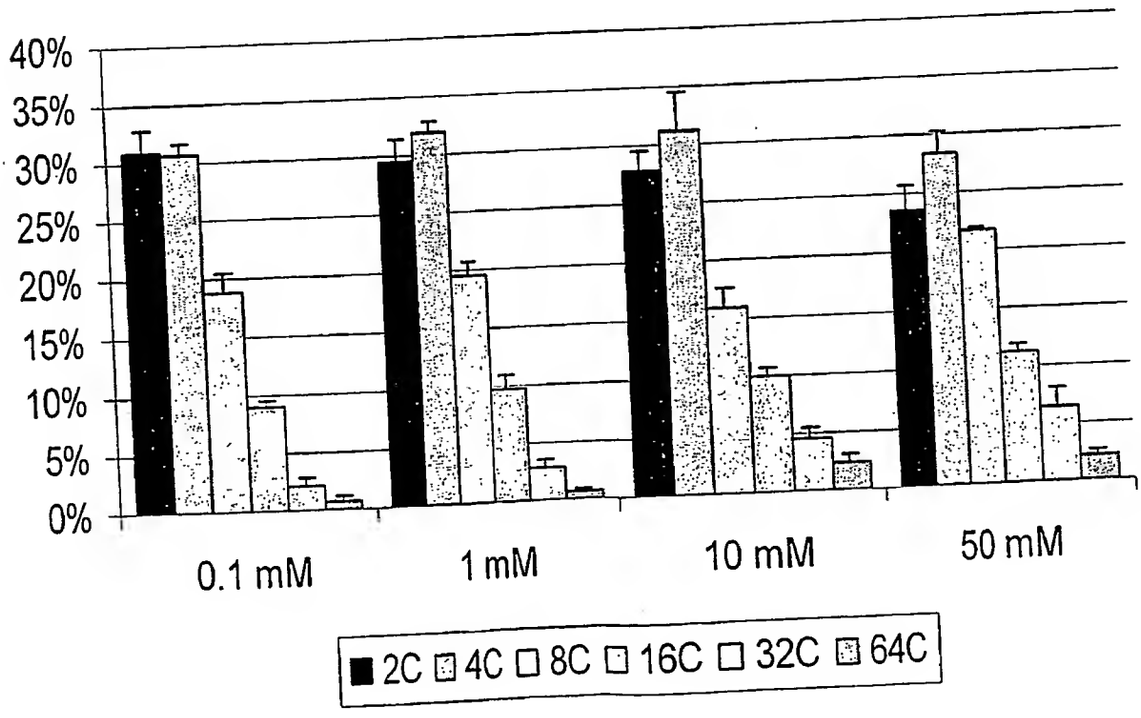
B


40%
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25%
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15%
10%
5%
0%
0.1 mM
1 mM
10 mM
50 mM
2C 4C 8C 16C 32C 64C

FIGURE 3

**MATDB - entry At1g57680 from contig t8l23**
(Chromosome 1 / BAC clone T8L23 / sequence database accession EMBL:AC079733) mips

Type: **gene/protein**
Code: **At1g57680**
Old code: **T8L23_15**
Title: **tative protein**
Contig: 3
Position: 32-54480 (C)

Notes

Classificat:

- known protein

**Functional Category**

- UNCLASSIFIED PROTEINS

**TargetP prediction**

- Targeted to secretory pathway
- TargetP score: 0.968
- TargetP reliability class: 2
- Probable signal sequence length: -

**TMHMM transmembrane prediction**

- Very likely to be a transmembrane protein (or have a signal peptide) (Exp number of AA in TMHs: 110)
- A transmembrane region could actually be a signal peptide (Exp number, first 60 AAs: 21)
- Orientation of N-terminal: external side (probability: 0.9)
- Transmembrane regions:
  - 40-62
  - 83-100
  - 138-160
  - 181-203
  - 213-235

**EMBL**

- AY072149

**mRNA matches: 1 found**

**Arabidopsis ESTs**

- found **10**
  AA585779; AI992654; AI998042; AV518701; AV538415; AV538372; AV541088; AV550688; AV550640; AV554579;



FIGURE 4

Full report

- Full report includes FST matches and external annotation... slow.

Protein properties

PEDANT and Interpro data are being recalculated. To access old PEDANT data, use the link in the left frame, but be aware that some protein sequences have been changed due to update of gene models based on cDNA data and PEDANT data may be outdated.

---

Click here to submit new information about this entry



FIGURE 4 (contin.)

*A. thaliana* - contig t8123 - entry At1g57680 mips

```
>P1;At1g57680
putative protein
MPLTKLVPDA FGVVTICLVA LLVLLGLLCI AYSFYFQSHV RKQGYIQLGY FSGPWIIRIT
FILFAIWWAV GEIFRLSLLR RHRRLLSGLD LRWQENVCKW YIVSNLGFAE PCLFLTLMFL
LRAPLKMESG ALSGKWNRDT AGYIILYCLP MLALQLAVVL SESRLNGGSG SYVKLPHDFT
RTYSRVIIDH DEVALCTYPL LSTILLGVFA AVLTAYLFWL GRQILKLVIN KRLQKRVYTL
IFSVSSFLPL RIVMLCLSVL TAADKIIFEA LSFLAFLSLF CFCVVSICLL VYFPVSDSMA
LRGLRDTDDE DTAVTEERSG ALLLAPNSSQ TDEGLSLRGR RDSGSSTQER YVELSLFLEA
EN*
C; Length 362 aa
C; Sequence At1g57680 was extracted from t8123
C; Fragment (54480-53392(C))
```



FIGURE 4 (contin.)

*A. thaliana* - contig t8123 - coordinates: 53392-54480 (C) mips

```
   1 ATGCCCCTGA CAAAATTAGT TCCCGATGCA TTCGGCGTTG TGACGATATG TCTAGTCGCT
  61 CTGCTAGTTC TTTTGGGTCT CCTTTGCATC GCTTACTCGT TCTATTTCCA GTCTCACGTT
 121 CGTAAGCAAG GCTATATTCA ACTTGGTTAC TTCAGTGGTC CCTGGATTAT CCGAATCACT
 181 TTCATTCTCT TTGCTATCTG GTGGGCTGTT GGTGAGATTT TTCGATTGAG TTTGTTGAGG
 241 CGTCACAGAA GGTTGTTGAG TGGGTTGGAT CTGAGATGGC AAGAAAACGT TTGCAAGTGG
 301 TACATCGTTT CCAATCTAGG ATTTGCGGAG CCTTGTCTCT TTCTGACTCT CATGTTTCTT
 361 CTGCGTGCTC CCTTGAAGAT GGAATCAGGG GCTTTGAGCG GAAAATGGAA CAGGGACACA
 421 GCAGGTTATA TTATTCTTTA TTGTCTCCCG ATGCTTGCTC TTCAACTTGC GGTTGTGTTG
 481 TCCGAGTCAC GCCTAAATGG TGGTAGTGGC TCTTATGTAA AGCTGCCACA CGACTTCACA
 541 AGAACGTATT CCCGAGTTAT TATTGATCAC GACGAGGTGG CCTTATGCAC ATATCCTCTA
 601 CTGAGTACCA TCCTTCTTGG TGTGTTTGCA GCCGTCCTAA CAGCTTACTT GTTCTGGCTT
 661 GGAAGGCAGA TACTGAAACT TGTCATTAAC AAGCGTTTAC AGAAGAGAGT ATACACTTTG
 721 ATATTCTCGG TCTCGAGTTT CCTTCCATTA AGGATTGTTA TGCTCTGTTT GTCGGTTCTC
 781 ACAGCAGCAG ACAAGATTAT ATTCGAAGCC CTTTCTTTCT TGGCCTTCCT CTCCCTCTTC
 841 TGCTTTTGCG TGGTATCCAT CTGCTTGCTT GTCTACTTCC CGGTTTCAGA TTCCATGGCC
 901 CTGAGAGGTC TAAGAGACAC AGATGATGAG GATACGGCTG TGACCGAAGA ACGCAGTGGT
 961 GCTCTGTTAC TTGCACCAAA CTCTTCACAA ACTGATGAGG GATTGAGCTT AAGAGGTCGG
1021 AGAGACTCGG GATCGTCTAC ACAGGAGAGG TATGTGGAAC TCAGCCTATT TCTGGAAGCT
1081 GAGAACTAA .
```



FIGURE 4 (contin.)

*A. thaliana* - contig t8l23 - coordinates: 53392-54480 (C) mips

```
   1 gtattatcta ttgagattct gtgtttaaag gttatgactt ggctttatgt atttcaatat
  61 tgttattgat ttgtgtgtta atcatcttaa tcttcaaggt tgctttagtt ttgagaaagg
 121 ttatgacttt ttaagatcta gggttaagat ggtttgatag gtctttgttt caaatttttg
 181 gtatatttgg taagtttttg attatttgtt gttttgttta gtttatgtag gtaacacgca
 241 tatcaagtgt taaagagtca agatcacaaa aagttctatc gggtgatctg ggctgctttc
 301 ttttgtaatc tasttgcaga aactttgctc tgacttggat agcttcgtaa aaggttcaat
 361 ctttctccgt ttttcatcaa tgagtagtaa ctaatctgga aatttgttgg gagagaaagg
 421 gcacattgca ctgctattgc tagagaacgt ttctgcatcc atgctggtag agagcatgcg
 481 tggatactgt gttttgggtg ATGCCCCTGA CAAAATTAGT TCCCGATGCA TTCGGCGTTG
 541 TGACGATATG TCTAGTCGCT CTGCTAGTTC TTTTGGGTCT CCTTTGCATC GCTTACTCGT
 601 TCTATTTCCA GTCTCACGTT CGTAAGCAAG GCTATATTCA ACTTGGTTAC TTCAGTGGTC
 661 CCTGGATTAT CCGAATCACT TTCATTCTCT TTGCTATCTG GTGGGCTGTT GGTGAGATTT
 721 TTCGATTGAG TTTGTTGAGG CGTCACAGAA GGTTGTTGAG TGGGTTGGAT CTGAGATGGC
 781 AAGAAAACGT TTGCAAGTGG TACATCGTTT CCAATCTAGG ATTTGCGGAG CCTTGTCTCT
 841 TTCTGACTCT CATGTTTCTT CTGCGTGCTC CCTTGAAGAT GGAATCAGGG GCTTTGAGCG
 901 GAAAATGGAA CAGGGACACA GCAGGTTATA TTATTCTTTA TTGTCTCCCG ATGCTTGCTC
 961 TTCAACTTGC GGTTGTGTTG TCCGAGTCAC GCCTAAATGG TGGTAGTGGC TCTTATGTAA
1021 AGCTGCCACA CGACTTCACA AGAACGTATT CCCGAGTTAT TATTGATCAC GACGAGGTGG
1081 CCTTATGCAC ATATCCTCTA CTGAGTACCA TCCTTCTTGG TGTGTTTGCA GCCGTCCTAA
1141 CAGCTTACTT GTTCTGGCTT GGAAGGCAGA TACTGAAACT TGTCATTAAC AAGCGTTTAC
1201 AGAAGAGAGT ATACACTTTG ATATTCTCGG TCTCGAGTTT CCTTCCATTA AGGATTGTTA
1261 TGCTCTGTTT GTCGGTTCTC ACAGCAGCAG ACAAGATTAT ATTCGAAGCC CTTTCTTTCT
1321 TGGCCTTCCT CTCCCTCTTC TGCTTTTGCG TGGTATCCAT CTGCTTGCTT GTCTACTTCC
1381 CGGTTTCAGA TTCCATGGCC CTGAGAGGTC TAAGAGACAC AGATGATGAG GATACGGCTG
1441 TGACCGAAGA ACGCAGTGGT GCTCTGTTAC TTGCACCAAA CTCTTCACAA ACTGATGAGG
1501 GATTGAGCTT AAGAGGTCGG AGAGACTCGG GATCGTCTAC ACAGGAGAGG TATGTGGAAC
1561 TCAGCCTATT TCTGGAAGCT GAGAACTAA a atcgccaaag gctgttccta ttggctttt
1621 ggcaatgtac agattcctgg tgaaacaagc agagagagag ggataaagag tttaagtatg
1681 agaatatgtt tgcgcaaaaa aaggcataat ttcagttttg tggcaaagac actttgactg
1741 taaaggaggg nttaaggggg tttactcttg tgagggtttg ttgtttgaaa tgtttttctgc
1801 ttgatggatc atattttttgt acctttatta tgtgatcaat tttgatttag aaaaaaaaaa
1861 aaaaaacaat agccgtgaac atgagcatga ctttaaaaga taaatcagcc ttttacccta
1921 tattctttgg actcatgaac atgagacttc ataagaaaat tagagaaatt gtaccaagca
1981 aaacagccat atagtgttct aaacttccat gtcaattcga cctagacaaa tacacttatg
2041 acttcagaaa atttgacata attttaatat ttaaccaagt ttgtcaaga
```

Sequences of 5' leader, 3' trailer, and introns (when applicable) are printed in lowercase.



FIGURE 4 (contin.)

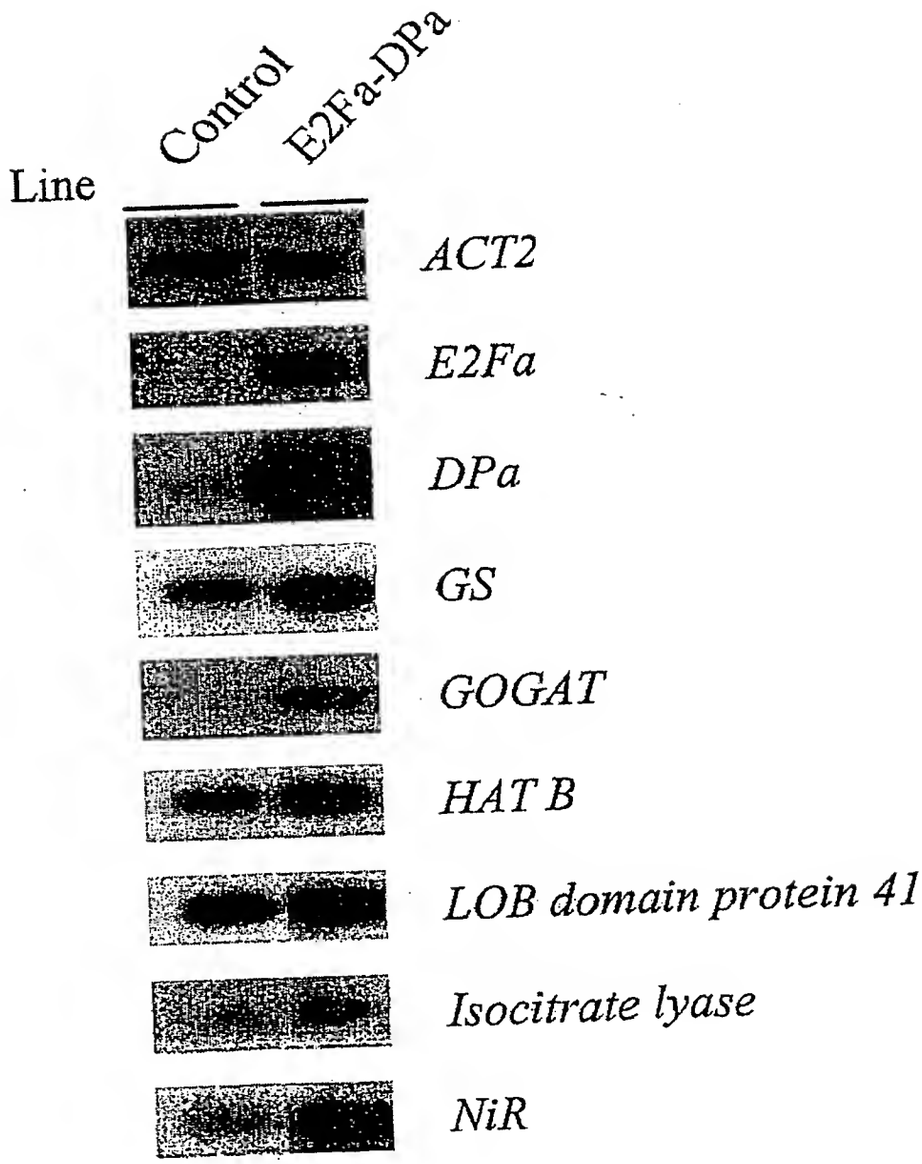
Control
E2Fa-DPa
Line
ACT2
E2Fa
DPa
GS
GOGAT
HAT B
LOB domain protein 41
Isocitrate lyase
NiR

FIGURE 5

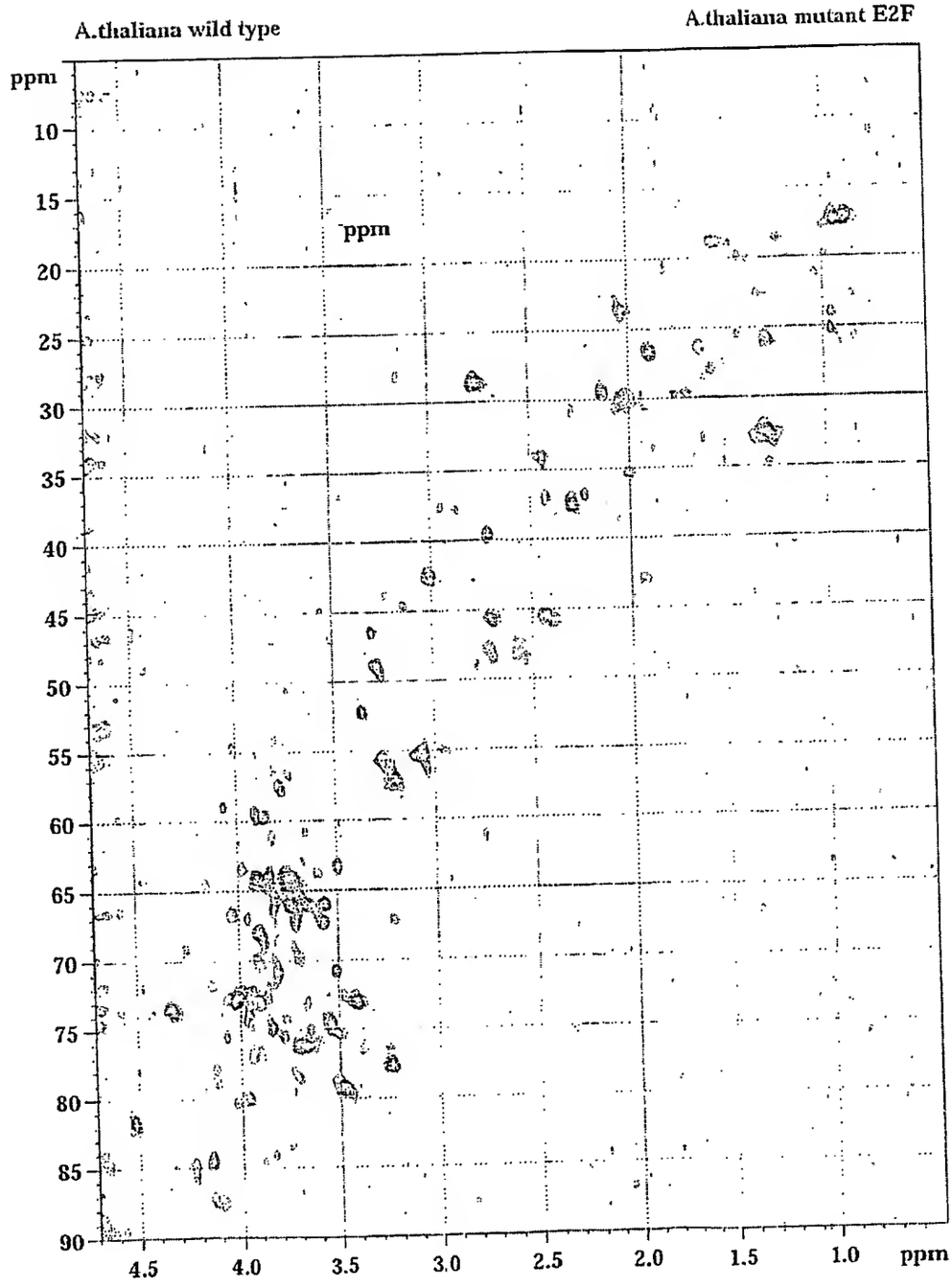
A.thaliana wild type
A.thaliana mutant E2F
ppm
ppm
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

FIGURE 6

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(75) Inventors/Applicants *(for US only)*: **INZE, Dirk** [BE/BE]; Driesstraat 18, B-9310 Moorsel-Aalst (BE). **DE VEYLDER, Lieven** [BE/BE]; Josef Boddaertdreef 23, B-9031 Drongen (BE). **VLIEGHE, Kobe** [BE/BE]; Ter Weibroek 51, B-9880 Aalter (BE).

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Puhlished:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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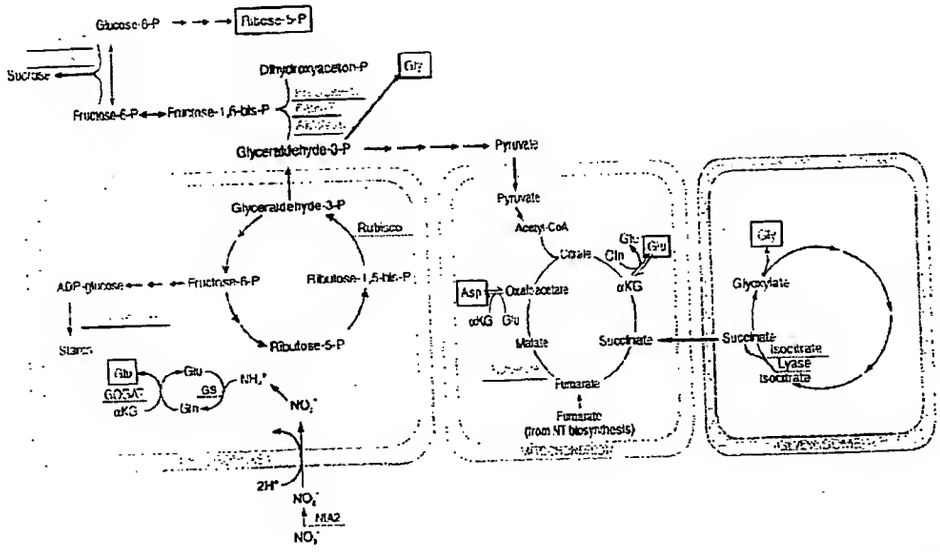
*[Continued on next page]*

(54) Title: IDENTIFICATION OF E2F TARGET GENES AND USES THEREOF




(57) Abstract: The present invention concerns a method for altering characteristics of a plant. The invention describes the identification of genes that are upregulated or downregulated in transgenic plants overexpressing E2Fa/DPa and the use of such sequences to alter plant characteristics. A preferred way for altering characteristics of a plant comprises modifying expression of one or more nucleic acid sequences and or modifying level and/or activity of one or more proteins, which nucleic acids and/or proteins are essentially similar to any one or more of SEQ ID NO 1 to 2755. Some of the genes identified in the present invention have an E2Fa target consensus sequence in their 5' upstream region. The identified genes play a role in a variety of biological processes, such as DNA replication, cell wall biosynthesis, nitrogen and/or carbon metabolism, transcription factors etc.

WO 2004/035798 A3

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/11658

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/82 C07K14/415 A01H1/04 A01H5/00 A61K31/713
A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 1 033 405 A (CERES INC)<br>6 September 2000 (2000-09-06)<br>page 341, line 11 - line 13; sequences<br>72617,72618<br>claims 1-34 | 1-16,30,<br>32,36,37 |
| L | DATABASE EMBL [Online]<br>18 October 2000 (2000-10-18),<br>ALEXANDROV N. ET AL.: "Arabidopsis<br>thaliana DNA fragment SEQ ID NO:72617"<br>XP002283128<br>Database accession no. AAC52840<br>L: document disclosing SEQ ID NO:72617 of<br>patent EP 1 033 405<br>the whole document | 1-16,30,<br>32,36,37 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 7 June 2004 | 01 09 2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Blanco Urgoiti, B |

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/11658

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| L | DATABASE EMBL [Online]<br>ALEXANDROV N. ET AL.: "Arabidopsis thaliana protein fragment SEQ ID NO:72618"<br>XP002283129<br>Database accession no. AAG56488<br>L: document disclosing SEQ ID NO:72618 of patent EP 1 033 405<br>the whole document<br>----- | 1-16,30, 32,36,37 |
| X | DATABASE EMBL [Online]<br>XP002283411<br>Database accession no. AC079733<br>nts 53131-54759<br>protein-id:AAG50748.1<br>page 8<br>----- | 5-11,16, 30,32, 36-39 |
| X | VEYLDER DE L ET AL: "Control of proliferation, endoreduplication and differentiation by the Arabidopsis E2Fa-DPa transcription factor"<br>EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB,<br>vol. 21, no. 6,<br>15 March 2002 (2002-03-15), pages 1360-1368, XP002227182<br>ISSN: 0261-4189<br>cited in the application<br>the whole document<br>----- | 1-4, 12-16,25 |
| A | VANDEPOELE K ET AL: "Genome-wide analysis of core cell cycle genes in Arabidopsis"<br>PLANT CELL, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US,<br>vol. 14, no. 4, April 2002 (2002-04), pages 903-916, XP002259203<br>ISSN: 1040-4651<br>cited in the application<br>----- | |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/11658

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 19 relates to an essentially biological process for the production of plants (Rule 39.1(ii) PCT)

2. [X] Claims Nos.: 26
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-39 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claim 19 relates to an essentially biological process for the production of plants (Rule 39.1(ii) PCT)

---

Continuation of Box I.2

Claims Nos.: 26

Claim 26 refers to enzymes and/or pharmaceuticals produced using the transgenic plants of claims 12 to 14 without giving a true technical characterization. Moreover, no such compounds are defined the application. In consequence, the scope of said claim is ambiguous and vague, and itsr subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such purely speculative claim whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-39 (all partially)

Method to alter plant characteristics modifying the expression of the gene of SEQ ID NO:1 or the protein level of SEQ ID NO:2; the nucleic acid of SEQ ID NO:1; method for makeing a transgenic plant introducing the DNA of SEQ ID NO:1; transgenic plants; host cells; methods for plant breeding; the DNA of SEQ ID NO:1 or the protein of SEQ ID NO:2 comprised in growth regulators, in therapeutic/diagnostic compositions, in kits or in food products; use of SEQ ID NOs:1 or 2 as markers; regulatory elements and promoters of the DNA of SEQ ID NO:1.

---

Invention 2: claims 1-39 (all partially)

As invention 1 but related to the DNA of SEQ ID NO:3 and the polypeptide of SEQ ID NO:4.

---

Invention 3 to 1368: Claims 1-39 (all partially)

Invention 3 being as invention 1 but related to SEQ ID NOs:5 and 6;...invention 1368 being as invention 1 but related to SEQ ID NO:2755.

---

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/11658

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 1033405 A | 06-09-2000 | CA 2300692 A1 | 25-08-2000 |
| | | EP 1033405 A2 | 06-09-2000 |